An Investigation into the Relationships between Synaptic Biochemistry, Clinical Symptoms and Pathology in the Lewy body dementias and Alzheimer's disease.

Whitfield, David Robert Edward

Awarding institution:
King's College London

An Investigation into the Relationships between Synaptic Biochemistry, Clinical Symptoms and Pathology in the Lewy body dementias and Alzheimer's disease.

David Robert Edward Whitfield

Thesis submitted for the degree of Doctor of Philosophy

King's College London

Wolfson Centre for Age-Related Diseases

School of Biomedical Sciences

King's College London

ABSTRACT

Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) are, combined, the second most common type of dementia in the elderly but remain both poorly understood and researched. Diagnosis is based upon clinical symptoms yet there is considerable overlap between DLB, PDD and Alzheimer's disease (AD), in terms of clinical presentation and pathology. Synapses are critical for neuronal communication and form the basis of memory and behaviour. The loss of zinc transporter 3 (ZnT3) has been implicated in age-related cognitive decline (based upon work in ZnT3-knockout mice), an observation corroborated by reports of reduced cortical zinc and ZnT3 in the brains of individuals with AD.

This project utilised a cohort of AD, PDD, DLB and control brains with cognitive data and semi-quantitative scores for key behavioural symptoms (depression, agitation, hallucinations, persecution) and the principal pathologies (amyloid-beta, tau and alpha-synuclein). Semi-quantitative Western blotting was used to investigate key synaptic proteins; zinc transporter 3, PSD95, beta-III-tubulin and synaptophysin in three cortical regions.

The major findings of this project were the occurrence of a loss of regulation of synaptic zinc, which predicted cognitive decline, depression and severity of amyloid-beta, tau and alpha-synuclein pathology in different cortical areas. Differences were also demonstrated in synaptic biochemistry between DLB and PDD cases, with PDD cases having a greater synaptic dysfunction.

Important outcomes of this study include the potential for zinc modulation as a new target for the treatment of depression and cognitive decline in LBD, possibly through a modification of pathology, and the potential for synaptic proteins to be utilised as biomarkers for the differentiation of DLB and PDD.

Acknowledgements

I am very grateful to my supervisors Professor Paul Francis Dr Tibor Hortobágyi Professor Dag Aarsland for their input, advice, encouragement and support throughout my PhD.

I would like to express my thanks to all of my friends and colleagues at the Wolfson Centre for Age-Related Diseases throughout the years, for making it such a stimulating and enjoyable environment to work in. I am indebted to all those colleagues who were specifically involved with this project. In no particular order these are; Dr David Howlett (whom I would like to thank for his considerable assistance with immunohistochemistry training and staining for semi-quantitative pathology scores and for being an unofficial 2nd supervisor/voice of wisdom!), Sindoo Rangarajan (who likewise contributed to the staining for semi-quantitative pathology scoring), Professor Clive Ballard (for advice in my transfer viva and throughout the project and for considerable assistance and advice in developing the semi-quantitative behavioural scores), Dr Antigoni Ekonomou (who likewise provided helpful advice in my transfer viva), , Dr Emma Jones (for advice and support in the lab, office and pub!), Dr Martin Broadstock (another voice of wisdom in the Francis lab!), Dr Julie Vallortigara (for her considerable efforts in assimilating the semi-quantitative behavioural scores, for help in tissue preparation, and in making the lab organised and tidy!), Amani Alghamdi (who likewise contributed a lot to the preparation of tissue for this project), Dr Natasha Bajic (for teaching me most of the lab techniques I acquired in this project) and everyone else on the floor (Byron, Ed, Talisia, Claire, Liz, Jack, Sean, Prav) for keeping lunchtimes a highlight of the day and for introducing me to the Blue Eyed Maid!

I would like to express my thanks to my colleagues associated with this project at the various brain bank centres; without their time and assistance in providing both the brain tissue and the data associated with it, this project would not have been possible.

In Oxford these were;

Professor Margaret Esiri, Dr Katherine Joachim, Carolyn Sloane, Dr Laura Parkkinen, Dr Olaf Ansorge, Sharon Christie and Grzegorz Agacinski.

In Newcastle these were;

Professor John O'Brien, Professor Alan Thomas, Dr Johannes Attems, Mary Johnson,

At the MRC London Neurodegenerative Diseases Brain bank these were;

Dr Claire Troakes, Richard Hudspith, Dr Safa Al-Sarraj, Dr Tibor Hortobágyi

I would like to give additional thanks to Dr Troakes for her assistance in teaching immunohistochemical techniques, and in organising the cohort (in particular for searching the comprehensive paper records of patients clinical data).

Additionally I would like to acknowledge Dr Tibor Hortobágyi and Dr Johannes Attems for undertaking the semi-quantitative scoring of pathology and Prof Dag Aarsland for his part in producing the semi-quantitative scores of behaviour.

My thanks to Dr Stephen Newhouse for providing an insightful and detailed plan and oversight of the statistical analysis conducted for this project.

I am very grateful to my funder the Alzheimer's society and in particular to the network volunteers who provided lay person mentoring to my project; Fay Andrews, Christine Cooper, Jean Town and John Harding.

I am very grateful to Hilary Davies (a Royal Literary Fund Fellow based at KCL) for advice on writing and grammar.

Finally, my thanks to my family and friends outside the Wolfson without whose support my journey here would not have begun.

List of Abbreviations

(f)MRI – (functional) magnetic resonance imaging

(s)Aβ – (soluble) amyloid beta

5-HT – 5-hydroxytryptamine

AChEI – acetyl cholinesterase inhibitors

AD – Alzheimer's disease

AMPA - α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate

AP3 – adaptor protein 3

APOe – apolipoprotein e

APP – amyloid precursor protein

αsyn – alpha-synuclein

ATP – adenosine triphosphate

BA(x) – Brodmann area

BDR – brains for dementia research

BSA – bovine serum albumin

βsyn – beta synuclein

Btub – β-III-tubulin

CAA – cerebral amyloid angiopathy

CERAD – consortium to establish a registry for Alzheimer's disease

ChAT – choline acetyltransferase

DBS – deep brain stimulation

DLB – dementia with Lewy bodies

DTI – diffusion tensor imaging

ELISA – enzyme-linked immunosorbent assay

FA – fractional anisotropy

FDG-PET – Fluorodeoxyglucose positron emission tomography

FTD – fronto-temporal dementia

GABA –gamma-aminobutyric acid

GBA – glucocerebrosidase

GWAS – genome-wide association study

IgG – immunoglobulin G

IHC – immunohistochemistry

IPL – inferior parietal lobule

LB – Lewy bodies (bLB/cLB) brainstem or cortical Lewy bodies

LBD – Lewy body dementias

LRRK2 – leucine-rich repeat kinase 2

LTD – long-term depression

LTP – long-term potentiation

MAGUK – membrane associated guanylate kinases

MAP – microtubule associated protein

MCI – mild cognitive impairment

MMSE – mini mental state examination

MT – mitochondria

MWM – molecular weight marker

NBT-BCIP - nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3`-indoyl-phosphate-p-toluidine salt

NFT – neurofibrillary tangles

NMDA – *N*-methyl-D-aspartate

NPI – neuropsychiatric inventory

PBST – phosphate-buffered saline with tween

PD – Parkinson's disease

PDD – Parkinson's disease dementia

PET blot – paraffin embedded tissue blot

PFC – prefrontal cortex

PiB – Pittsburgh compound B

PIGD – postural-instability gait difficulty

PMD – post-mortem delay

PSD – post-synaptic density

RCT – randomised clinical trial

REM – rapid eye-movement sleep

SDS – sodium dodecyl-sulphate

SDS-PAGE – sodium dodecyl-sulphate polyacrylamide gel electrophoresis

SNARE - soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors

SNP – single nucleotide polymorphism

SPECT – single-photon emission computed tomography

SPP - synaptophysin

SSRI – selective serotonin reuptake inhibitor

TBS – tris-buffered saline

TBST – tris-buffered saline with tween

TD – tremor dominant

ZnT3 – zinc transporter 3

List of Tables

List of Figures

List of Appendix Tables

Table of Contents

1 Introduction

1.1 Neurodegeneration and Dementia

1.1.1 Background

Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD), collectively described as Lewy body dementias (LBD), have very similar pathology yet a subtly different clinical presentation. There is debate about the prevalence of DLB, ranging from 7% of all dementia cases (Kester and Scheltens, 2009) to the higher estimate of around 15% (Aarsland et al., 2008); however most studies agree DLB is the second most common type of dementia. Again, there are differing estimates of PDD prevalence, between 40-60% of Parkinson's disease (PD) patients at age 70 ultimately develop PDD, this increases to 80-90% at age 90 (Buter et al., 2008; Kester and Scheltens, 2009); in another study over a 20 year period around 80% of PD patients developed dementia (Hely et al., 2008). However a more conservative estimate provided by a comprehensive review of PDD prevalence studies estimates 24% of PD patients to have dementia (Aarsland et al., 2005c). Furthermore it is highly probable that most – if not all- PD patients would eventually develop PDD based upon an 8 year prospective study in which over 75% of PD patients developed PDD (Aarsland et al., 2003).

Both DLB and PDD are characterised by pathological deposition of aggregated protein into intracellular inclusions called Lewy bodies (LB) and dystrophic neuritis also called Lewy neurites. Clinically DLB and PDD share the core symptoms of fluctuating cognition (referring to considerable variances over a short time in abilities such as attention and language), visual hallucinations and parkinsonism yet can be thought of as existing on a spectrum. Other common symptoms such as psychosis, aggression/agitation, depression and sleep disturbances are responsible for a great amount of carer burden and represent an unmet treatment need (McKeith et al., 2004; McKeith et al., 2005).

1.1.2 Symptoms

1.1.2.1 DLB, PDD and AD overview

The central clinical feature required for a diagnosis of probable or possible DLB, as defined by the third report of the DLB consortium is 'Dementia defined as progressive cognitive decline of sufficient magnitude to interfere with normal social or occupational function'; this can be accompanied by an increasing degree of memory loss and worsening attention, executive function and visuospatial function (McKeith et al., 2005). McKeith and colleagues outline a number of other clinical features to assist in diagnosis DLB. Symptoms that are termed 'core features', two of which are required for a diagnosis of probable DLB and one for possible DLB, are 'fluctuating cognition with pronounced variations in attention and alertness, recurrent visual hallucinations that are typically well formed and detailed, and spontaneous features of parkinsonism' (McKeith et al., 2005). Additionally there are a number of 'suggestive' features (REM sleep behaviour disorder, neuroleptic sensitivity and low dopamine transporter uptake in the basal ganglia) and 'supportive' features (see subsequent section on other symptoms) that contribute towards diagnosis.

Whilst cognitive and functional decline remain the core features of dementia a wide variety of behavioural symptoms can present in many dementia cases before or after the onset of the aforementioned core symptoms. Such behavioural symptoms can represent a more significant problem to carers and patients than the typical decline in activities of daily living and in cognition that occurs in dementia (McKeith et al., 2005). Generally behavioural symptoms follow the same pattern of a gradually increasing severity as the disease progresses however there is variation in this; some behaviours such as hallucinations and REM sleep behaviour disorder do not consistently obey this trend – but rather decline or disappear as the disease progresses (Ballard et al., 1997; Boeve et al., 2004; Gauthier et al., 2010).

Cognitive symptoms in PDD and DLB are predominantly confined to attention, constructional, memory, visuospatial and executive domains (Emre et al., 2007). Behavioural and neuropsychiatric symptoms include hallucinations, which are common in PDD (around 45%) and even more so in DLB (up to 80%), and tend to coexist with delusions. Mood disturbances are another frequent symptom

in all three dementias, these include depression anxiety and apathy. Apathy in particular has been reported to be present in up to 80% of DLB patients and around 50% of AD. In general, behavioural and neuropsychiatric symptoms are less frequent and/or severe in PDD. PDD is also characterised by motor symptoms, as is DLB following the onset of dementia. The severity tends to be similar but not all DLB cases present with motor symptoms (McKeith et al., 2004). These typically present as progressive bradykinesia, rigidity, postural instability and tremor (Dickson, 2012).

Alzheimer's disease is defined by a gradual onset of the following symptoms; worsening cognition (in particular learning and recall, word finding, spatial cognition and executive dysfunction) (McKhann et al., 2011).

See subsequent sections for further details on the symptoms of particular interest to this project.

1.1.2.2 Fluctuating Cognition

Fluctuating cognition, although a core feature of DLB, is by no means exclusive to DLB but is a reported in other dementias – notably in Alzheimer's disease (AD) (Bradshaw et al., 2004). Bradshaw and colleagues undertook a small study comparing fluctuations between DLB and AD patients in which they described several qualitative differences in fluctuations between the two dementias based upon interviews with caregivers and patients (Bradshaw et al., 2004). Fluctuations in DLB were typically characterised by a greater variation in the abilities of the individual than in AD and this variation tends to occur over a shorter time interval - within the course of a single day whereas the AD patients in the study generally had fluctuations lasting for an entire day (Bradshaw et al., 2004). This is consistent with the consensus that fluctuating cognition is more frequent and of greater severity in DLB than AD (McKeith et al., 2005).

Additionally Bradshaw and colleagues observed that fluctuations in AD appeared to be triggered by context and environment – and that this tended to result in increased repetitiveness and forgetfulness; whereas in DLB no trigger to fluctuations was evident – suggesting an internal mechanism behind this feature. The resulting decline in cognitive ability was typified by reduced attention and awareness (Bradshaw et al., 2004).

However reliable and accurate assessment of fluctuations in dementia remains challenging with poor inter-rater reliability between scorers, despite the prevalence of fluctuating cognition, this is in part due to its inherent variability (Cummings, 2004; McKeith et al., 2005).

1.1.2.3 Visual Hallucinations

Visual hallucinations in Lewy body dementias are usually complex and well formed (often featuring animals) and as a key symptom of DLB and PDD they represent an important tool for diagnosis and discrimination from other dementias (McKeith et al., 2005; Tröster, 2008). Deficits in functions related to the visual system may predispose individuals to visual hallucinations, Cagnin and colleagues recently demonstrated poor visual attention to be an independent predictor of visual hallucinations (Cagnin et al., 2012; Tröster, 2008). Cagnin and co-authors highlight the concord between this finding (Cagnin et al., 2012) and a recent model for the genesis of visual hallucinations proposed by Collerton et al. in which it was suggested that deteriorations in attention and visual perception generate hallucinations (Collerton et al., 2005). Visual hallucinations are unique amongst DLB and PDD symptoms in that a clear link to pathology in a specific brain region has been demonstrated. Harding et al. showed a strong positive relationship between Lewy bodies in the temporal cortex and the presence of visual hallucinations – with early onset of hallucinations being predicted by a density of Lewy pathology in the parahippocampal and inferior temporal cortices (Harding et al., 2002).

In terms of the neurochemistry of hallucinations; deficits in cholinergic neurotransmission in the temporal cortex may be partially responsible for hallucinations in LBD – two studies examining choline acetyltransferase (ChAT) levels found a significant relationship between reduced levels of ChAT and visual hallucinations in part of the visual association area (the perirhinal cortex - BA36) (Ballard et al., 2000; Perry et al., 1990).

1.1.2.4 Parkinsonism

Parkinsonism in DLB broadly resembles that found in PD (and therefore by extension PDD) (McKeith et al., 2005). One difference between parkinsonism in DLB and PDD is a difference in the predominance of the two forms – postural-instability gait difficulty (PIGD) and tremor dominant (TD); in a study of around 100 patients the former was the more common in DLB and PDD whereas there was an equal distribution of the two forms of parkinsonism in non-demented PD patients (Burn et al., 2003). PIGD and TD were formalised as the primary subtypes of clinical presentation of parkinsonism in 1990 in a cohort study by Jankovic and colleagues (Jankovic et al., 1990). The PIGD subtype - in addition to the instability of posture and difficulties with gait implicit in the name - is characterised by a greater severity of bradykinesia and impairment of motor function, intellectual faculty and daily activities of living than TD; furthermore PIGD is associated with a more accelerated progression and earlier onset of disease (Jankovic et al., 1990).

1.1.2.5 Other Symptoms

REM sleep behaviour disorder is a termed a 'suggestive feature of DLB' according to the 3rd report of the DLB consortium (McKeith et al., 2005) due to its rarity in non-synucleinopathy dementias and prevalence of 50% in LBD (Boeve et al., 2004). Typically presentation consists of aggressive, and sometimes complex, movement and vocalisation accompanied by vivid and distressing dreams during sleep (McKeith et al., 2005). It has been suggested REM sleep behaviour disorder is a precursor to LBD as in a study of 27 non-demented individuals with REM sleep behaviour disorder around 63% subsequently developed DLB or PDD (Claassen et al., 2010).

Of the LBD symptoms termed 'supportive' only those that feature in the data for this study will be dealt with in detail here; these are depression, delusions and agitation.

1.1.2.6 Depression

Depression is common in PD, DLB and PDD and may be up to twice as common as it is in AD; as demonstrated by a comprehensive study based upon Geriatric Depression Scale scores from a cohort of 192 patients – half with AD and half with DLB (Yamane et al., 2011). This is supported by a

similar finding in another study – in which 50% of participants with dementia showed signs of depression and that depression was significantly more frequent in DLB than AD (Fritze et al., 2011b). However there remains some debate as to the relative frequencies of depression across the dementias, a notably large study involving 921 patients by Caputo et al. found no difference in the rates of depression between AD and DLB (Caputo et al., 2008).

Persistence of depression is another factor contributing to the clinical picture in DLB and is likewise more pronounced than it is in AD (Fritze et al., 2011a).

The question of the origin of geriatric depression is a complex one. There may be several factors independently linked to depression found in dementia. Depression alone is – according to the World Health Organisation – the leading cause of disability in the world (Reddy, 2010) and is an established risk factor for dementia. Depression has been shown to worsen cognition, an effect which is likely to contribute to the development of dementia (Rapp et al., 2011); although the authors remark upon the limitation imposed by the poor diagnostic reliability of depression. Additional evidence for depression having a causative link to dementia is provided by a study showing a high ratio of plasma Aβ 40 to Aβ 42 to be associated with depression and reduced cognition in a subset of depressed individuals participating in the study which the authors speculate to represent a prodromal stage of AD (Sun et al., 2008).

Other factors such as excessive cortisol (hypercortisolemia) as a result of depression and stress activating the have been implicated in the genesis dementia. Hippocampal neurons appear to be particularly vulnerable to hypercortisolemia, which can result in decreased hippocampal volume and a consequent cognitive deficit (Byers and Yaffe, 2011).

An investigation comparing amyloid brain imaging (Pittsburgh compound B (PiB) retention – see brain imaging section for discussion) in individuals with mild cognitive impairment (MCI) with and without depression further supports the notion that depression is a prodromal marker for dementia.

Butters et al. observed normal PiB binding when cognition was unperturbed regardless of the depressive state of the individual, yet 50% of participants with MCI and depression showed PiB retention consistent with that found in AD (Butters et al., 2008). Furthermore, it has been demonstrated that increased tau and Aβ pathology in the hippocampus was associated with a history of depression in AD patients, although this did not hold true for other cortical regions, this probably contributed to the accelerated cognitive decline reported in these individuals (Rapp et al., 2006). This link to the pathology and biochemistry of AD is crucial in confirming that the high incidence of depression in dementia is not simply an artefact of the prevalence of the two conditions in the elderly.

Structural changes occur in brain regions associated with cognition (prefrontal cortex and hippocampus) in chronic and late-onset depression (Ballmaier et al., 2008; Xekardaki et al., 2012). Once these imaging methods become more established they could prove invaluable as a screening and diagnostic tool for the early stages of dementia.

However, there is a counter argument that it is dementia that generates and drives depression; individuals at an early stage of dementia are aware of their condition and prognosis. This can have a profound psychological impact including a highly negative self-perception leading to depression (Lahr et al., 2007). Finally other factors may obscure the picture such as sleep disturbance and 'sub-optimal effort' as a consequence of depression artificially reducing the score on cognitive tests (Weisenbach et al., 2012).

1.1.2.7 Agitation and Aggression

Agitation and aggression are two intrinsically interwoven symptoms that can present in LBD and AD. They are associated with an increased risk of adverse events (such as injury) to the patient and increased distress to both carer and patient, and as a direct consequence are linked to earlier institutionalisation (Ballard et al., 2009).

Aggression can manifest as verbal and/or physical assault, frequently whilst personal care is being administered; whereas agitation typically presents as restlessness and anxiety-related behaviours such as hand-wringing and pacing. Both agitation and aggression have been linked to more rapid progression of dementia, as are many of the physical issues associated with the later stages of dementia such as dehydration, infections and pain, which are in turn established triggers of agitation and aggression (Hall and O'Connor, 2004). Estimates of the prevalence of agitation and aggression in dementia vary immensely from 10% to 100%, probably due to bias introduced by the setting of the study and care-giver interviews (a common source of information on psychiatric symptoms) (Sachs, 2006). Other factors that can initiate or compound agitation and aggression include environment related stress (eg. noise) and sensory impairment (eg. hearing loss) (Hall and O'Connor, 2004). Thus, by addressing elements such as these, successful management of agitation and aggression can be achieved though non-pharmacological means (Ballard et al., 2009).

The majority of studies examining the relationship between cognitive status and agitation/aggression have demonstrated an inverse relationship (negative correlation) (Hall and O'Connor, 2004). When Welsh and colleagues dissected the cognitive impairment they reported that – based upon CAMCOG scores - language impairment was the greatest predictor of aggression (Welsh et al., 1996), something that has also been observed in younger psychiatric patients; the proposed explanation was linked to the resultant frustration from an inability to communicate (Hall and O'Connor, 2004).

Imaging brain metabolism (FDG-PET – see imaging section) in AD patients suggests frontal and temporal lobe hypofunction (in the guise of reduced metabolic activity) to be significant contributors to agitation and aggression (Sultzer et al., 1995). This is supported by two studies, one – Norton et al. - using a behavioural test specifically designed to assess frontal dysfunction – the Frontal Lobe Personality Scale – which found a similar link between frontal lobe dysfunction and agitation and

aggression; whereas Tekin and colleagues demonstrated this link through the Neuropsychiatric inventory (Norton et al., 2001; Tekin et al., 2001).

Whilst anatomical correlates have been established for agitation and correlation, knowledge of the neurochemistry of agitation and aggression in dementia remains muddled but likely involves the prefrontal cortex. Indeed it has been shown that a reduction in alpha 1 adrenoceptors in the prefrontal cortex is associated with increased aggression in AD patients (Sharp et al., 2007). Other studies have shown increases in noradrenaline levels and heightened sensitivity to noradrenaline in AD patients – perhaps representing a compensatory mechanism in response to the decrease in adrenoceptors (Lindenmayer, 2000). Decreases in GABA and reductions in dopamine may also be involved in mediating agitative and aggressive behaviour in dementia (Sachs, 2006).

Investigation and assessment of agitation and aggression remains challenging due to a host of issues such as poor representation in clinical trials and studies (because of non-compliance of individuals with agitation and aggression as a direct result of this symptom), multiple criteria for defining agitation and aggression and coincidence with other psychiatric symptoms (complicating distinguishing cause and effect of individual symptoms) (Sachs, 2006). Two established tools for assessing agitation are the Neuropsychiatric Inventory (see cognitive and behavioural tests section) and the Cohen-Mansfield Agitation Inventory (Cohen-Mansfield et al., 1989). In a comparison of the inter-rater and test-retest reliability of these two scales the Cohen-Mansfield Agitation Inventory was shown to be superior (Zuidema et al., 2011).

1.1.2.8 Persecution/Delusions

Up to 45% of AD patients have been reported to experience delusions which often centre around suspicions of theft, infidelity and persecution (Gauthier et al., 2010) and this is also a commonly reported symptom ‘supportive’ of a diagnosis of DLB and PDD (McKeith et al., 2005). Studies have reported prevalence rates of delusions in DLB as high as 78% - and even the more conservative estimates of 57% are considerably higher than both AD and PDD – delusions in the latter have been

reported to occur in around 25 to 30% (Emre et al., 2007). Furthermore delusions tend to be persistent – Steinberg et al. found 65% of demented individuals suffered delusions for more than 1 year at follow-up (Steinberg et al., 2004).

Ballard and colleagues found delusions to be linked to an increase in M1 muscarinic receptor binding in BA36, the same region implicated in visual hallucinations. This provides a mechanistic basis for the coincidence of visual hallucinations and delusions in many patients (Ballard et al., 2000). Comparisons of SPECT imaging in dementia patients with and without delusions of theft and persecutions have highlighted another brain region – the frontal cortex – as involved in these symptoms (Nagahama et al., 2010).

1.1.3 Cognitive and Behavioural Tests

1.1.3.1 NPI

The Neuropsychiatric Inventory (NPI) is one of the most commonly used assessments of behavioural symptoms and disturbances in dementia. It takes the form of screening questions and in-depth 'sub-questions' asked of a carer familiar with the patient ideally on a monthly basis. Twelve symptoms are covered; hallucinations, delusions, agitation/aggression, dysphoria/depression, anxiety, irritability, disinhibition, euphoria, apathy, aberrant motor behaviour, sleep and night-time behaviour changes and appetite and eating changes (Cummings, 1997; Cummings et al., 1994). The score is a multiplication of the intensity of the symptom by the frequency. Carers represent a superior source of information on these symptoms to patients as patients are prone to forgetting symptoms; an issue that can be compounded by patients with severe dementia having difficulty in understanding relevant questions. Thus it is the carer who is asked to rate the severity and frequency of any of the listed behaviours which has occurred in the given time-frame, in addition to the degree of distress caused to the carer by the behaviour in question (Cummings, 1997).

The NPI has been shown to perform at least as well as two other behavioural tests (BEHAVE-AD and the Hamilton rating scale for depression) in scoring symptoms in a group of patients and carers, half of which had AD and half other dementias (Cummings, 1997). Notable advantages of the NPI include the wide range of behaviours assessed, its relative ease of scoring (due to the observable nature of each behavioural domain) and speed of administration (a consequence of the screening questions allowing the avoidance of the documentation of behaviours not present) (de Medeiros et al., 2010). Furthermore, the behaviours featured in the test are specific to dementia, some - such as euphoria and disinhibition – being uncommon in AD assist in distinguishing AD from other dementias. The NPI resolves a similar issue with regards to determining if depression is a symptom of dementia or has occurred independently (Cummings, 1997).

Drawbacks of the NPI include the dependence on the caregiver for information as there clearly is a possibility for bias or distortion of the scoring depending on the caregiver's background and

relationship to the patient. Another is a lack of sensitivity in distinguishing mild and severe dementia (de Medeiros et al., 2010).

1.1.3.2 MMSE

The Mini Mental State Examination (MMSE) is a questionnaire designed in 1975 by Folstein and colleagues (Folstein et al., 1975) for the purpose of providing a rapid and easily delivered numerical assessment of cognitive function. A testament to the success of the MMSE is the fact that it is now the most widely used tool for evaluating cognition across multiple languages and psychiatric disorders, in particular dementia (Bak and Mioshi, 2007). Despite this utility the MMSE is not recommended by Mckeith et al. as being sufficient to diagnose DLB as it was unable to detect crucial features such as fluctuating cognition and visual hallucinations (McKeith et al., 2005). However, despite development of more complex and sensitive tests, the MMSE is still appropriate and accurate for screening MCI and AD from healthy cognition, as shown in a comparative study with two such cognitive tests (CAMCOG and Montreal Cognitive Assessment) conducted on over 300 AD patients and 140 individuals with MCI (Roalf et al., 2012).

It is sometimes of assistance to research to use MMSE scores (which can total 30 provided the subject's faculties such as vision and hearing enable an attempt at all questions) as a basis for categorising patients into 'cognitively normal', 'moderately impaired' or 'severely impaired' based upon scores of 24 or greater, 11 to 23 and below 11 respectively (Boller et al., 2002).

1.1.4 Biomarkers

1.1.4.1 PET and SPECT

Functional brain imaging techniques, primarily positron emission tomography (PET) and single-photon emission computed tomography (SPECT), are increasingly being used to assist with antemortem diagnosis in suspected dementia patients. Both PET and SPECT measure gamma radiation produced from radioactive tracer compounds introduced into the brain. PET imaging is used to provide a 3D image of cerebral glucose metabolism. PET compounds in routine use are typically radioactively labelled sugars, such as fluorodeoxyglucose (FDG), that emit positrons which interact with electrons to produce two photons travelling in opposite directions; this is instrumental in achieving the higher resolution of PET when compared to SPECT. Thus the gamma radiation from a region of brain tissue corresponds to the degree of sugar uptake and hence metabolism, from which a measure of brain activity can be gained (Bear et al., 2006; Broderick, 2005).

The uptake of SPECT compounds (eg hexamethylpropylene amine oxime – Tc-HMPAO) into brain tissue is proportional to the blood flow, which in turn is tightly linked to metabolism and brain activity; providing a 3D image of cerebral blood perfusion. However the gamma radiation recorded from a SPECT image is directly emitted by the compound causing a loss in resolution compared to PET imaging (Broderick, 2005).

Another important difference between PET and SPECT is the tracer compounds themselves; PET compounds have a shorter half life than typical SPECT compounds and so must be synthesized on site prior to a scan, escalating the cost of a PET scan. SPECT compounds, having a considerably longer half-life, do not suffer this drawback and consequent expense (Broderick, 2005).

PET studies in patients with DLB, PDD and PD have revealed much about the pattern of metabolic deficit across brain regions and can assist with the separation of these diseases, which remains a clinical challenge, in particular establishing what (if any) underlying characteristics PDD and DLB share.

All three neurodegenerative conditions tend to have reduced metabolic activity in parietal, frontal, anterior cingulate and occipital regions with the greatest deficit occurring in DLB (Yong et al., 2007). A relative preservation of metabolic levels in medial temporal cortices coupled with a reduction in the occipital cortices in DLB and PDD can distinguish these dementias from AD (Ishii et al., 1999). Furthermore DLB and PDD may be differentiated from PD by the greater deficit in parietal and frontal metabolism (Yong et al., 2007). Indeed Yong et al. suggest that the progression of metabolic deficit in the frontal and parietal regions may be a substrate for the progression of PD into PDD.

It is likely that clinical aspects of dementia can be attributed to decreases in metabolic activity in a specific brain region. Firbank et al. propose that metabolic deficits in the lateral parietal cortex in PDD may have some responsibility for visual hallucinations in PDD patients (Firbank et al., 2003). Likewise Yong et al. argue that decreased frontal activity may correspond to executive and visuospatial dysfunction, this is supported by the matching increased hypometabolism and executive dysfunction generally reported in DLB over PDD.

When assessing the current knowledge of metabolic profiles across brain regions in the Lewy body dementias evidence can be found for the theory of DLB and PDD existing on a spectrum rather than as discrete disease states (McKeith, 2009). These alterations in brain consumption of glucose and rates of regional blood perfusion have provided a considerable improvement to the differentiation and diagnosis of dementias.

PET imaging can be used to assess regional alterations in neurotransmitter receptors. When administered to a subject, radio-labeled fluorodopa is taken up into dopaminergic neurons at a rate proportional to *l*-amino decarboxylase activity, itself dependent on the number of dopaminergic synapses in that brain region (Broderick, 2005).

Differentiating PDD from PD is relatively easy compared to the far harder task of separating DLB from AD especially at an early stage if parkinsonism symptoms have not manifested and the

fluctuating cognition is subtle (Walker and Walker, 2009). Some loss of nigrostriatal dopaminergic neurons occurs prior to expression of a parkinsonism phenotype, this was corroborated by *ex vivo* ligand binding studies of the dopamine system which showed DLB cases to have around a 57% loss of dopamine nerve terminals whilst AD and control cases were not different to one another (Piggott et al., 1999). FP-CIT SPECT (a cocaine analogue marketed commercially as DaTSCAN by GE Healthcare) is increasingly used for *in vivo* imaging of the mid brain dopamine system and typically shows a similar profile of binding to that reported ex-vivo (Piggott et al., 1999), reduced ligand uptake in DLB, with and AD and controls not significantly different in uptake (Walker and Walker, 2009).

Clinical diagnosis of DLB following the consensus guidelines set out by McKeith and colleagues (McKeith et al., 2005) has less than perfect sensitivity and specificity hence a follow up confirmation of FP-CIT scan results with post-mortem diagnosis is the gold standard. The validity of this approach was supported by a study where all bar one case with abnormal FP-CIT were pathologically confirmed as DLB and none with normal FP-CIT were determined to be DLB, thus elegantly demonstrating the clinical utility of FP-CIT in diagnosing DLB (Walker et al., 2007). This is of great advantage in PD patients but less so in dementia patients where imaging of other neurotransmitter systems such as the cholinergic would be of greater interest yet there are no suitable radiolabelled ligands for any other neurotransmitter system.

Development of Pittsburgh compound B (PIB), a thioflavin analogue that binds selectively to Aβ, allowed in vivo imaging of Aβ for the first time (Klunk et al., 2004). It remains the most widely used experimental method of visualizing AD pathology in patients after it was first demonstrated that PIB uptake in AD was significantly higher than controls in a number of regions, notably in the prefrontal cortex (PFC), anterior cingulated gyrus, parietal and temporal cortices (Klunk et al., 2004; Quigley et

al., 2011). Imaging Aβ in this way offers the potential of a window to early disease events and progression of the disease, something that is generally denied to post-mortem studies.

PIB PET studies on AD cases corroborated the amyloid cascade hypothesis after follow up studies on patients showed PIB binding to plateau during disease progression in accord with the amyloid hypothesis proposition that amyloid accumulation is an early event and reaches a peak well in advance of the terminal stages of the disease (Quigley et al., 2011). PIB PET investigations have also demonstrated a lack of correlation between Aβ levels and cognitive performance and some studies have shown elevated PIB levels in control patients (Jack et al., 2008; Klunk et al., 2004; Quigley et al., 2011) which may indicate Aβ to be less dominant in driving cognitive deterioration than sometimes argued.

Investigations of PIB uptake in DLB and PDD cases have shown some DLB cases to resemble AD in that it is characterised by a higher Aβ burden than control and PDD – both of which were similar in their PIB uptake (Edison et al., 2008; Gomperts et al., 2008). This contrast between DLB and PDD was not accounted for by dementia severity; indeed Gomperts et al. propose that Aβ deposition in PD advances onset of cognitive decline resulting in the reduced interval between motor and cognitive symptoms necessary for diagnosis of DLB.

PIB does not bind to α-syn or tau; this allows identification of so called pure AD from dementias with little or no Aβ such as fronto-temporal dementia (FTD) and even PDD, but makes distinguishing AD from DLB harder. However the reported differences between DLB and PDD allow more confidence in separating these dementias than the 1 year rule established by the DLB consortium (McKeith et al., 2005). Regardless, there is a clear need for PET tracers that can selectively bind α-syn and tau to assist in differentiating AD and DLB.

1.1.4.2 MRI

The third major form of functional brain imaging employed in the diagnosis, management and research of dementia is functional magnetic resonance imaging (fMRI) – a development of magnetic resonance imaging (MRI). MRI takes advantage of the jump between different energy states that hydrogen atoms undergo when exposed to a magnetic field, this allows differences in the amount of hydrogen present to be visualised. Because white matter, grey matter, blood vessels and other anatomical features of the brain have differing compositions (fatty myelin sheaths, lipid membranes, water, plasma, etc), their respective hydrogen atom contents also differ and can be distinguished on this basis with the aim of computer software (Bear et al., 2006; Broderick, 2005).

Demyelination and lesions are particularly evident on MRI scans due to the loss of fat and increase in water these events cause (Bear et al., 2006). Before the advent of functional MRI, conventional MRI was used to provide basic anatomical images of the brain. These had their use in providing insights into atrophy of certain brain regions in dementia, indeed hippocampal atrophy remains a good predictor for AD (Murray, 2011).

fMRI takes advantage of the difference magnetic resonances of oxyhaemoglobin and deoxyhaemoglobin and utilises this to image metabolism via the demand and use of oxygen by neurons in different brain regions revealed by the type of haemoglobin found there (Bear et al., 2006).

Diffusion tensor imaging (DTI) is a new form of fMRI put to use in DLB and PDD studies which utilises the increase in mean diffusivity of water when neurodegeneration has damaged neuronal structure and brain tissue, in particular white matter tracts. This is referred to as fractional anisotropy (FA). A decrease in the directionally orientated diffusion of water corresponds to a decrease in FA (Medina and Gaviria, 2008; Watson et al., 2012).

As with many imaging studies there are conflicting reports from DTI investigations into LBD (due to low numbers of cases, patient variability and inconsistencies in diagnosis), but most of the evidence is for a reduction in FA in the inferior longitudinal fasciculus (involved in recognition of written language (Shinoura et al., 2013)), posterior cingulate gyrus (see brain regions section) and precuneal areas (instrumental in the retrieval of contextual episodic memory (Lundstrom et al., 2005)) (Watson et al., 2012). DLB is generally characterised by a more posterior reduction in FA, in particular the parieto-occipital regions, which could in part explain the visual disturbances common in DLB. AD tends towards a more generalised reduction in FA across the brain(Watson et al., 2012).

DTI corroborates evidence from SPECT and PET investigations showing precuneal and occipital hypo perfusion and metabolism (Colloby et al., 2002; Imamura et al., 1997). These regions are not associated with any great degree of grey matter loss (Middelkoop et al., 2001) leading Watson et al. to suggest that DLB is characterised by white matter loss and therefore synaptic dysfunction as opposed to loss of cell bodies (Watson et al., 2012).

1.1.5 Treatment

1.1.5.1 Treatment of motor symptoms

Treatment of Lewy body diseases has the three aims of optimising motor and cognitive function whilst minimising unwanted symptoms of depression, hallucinations, delusions, anxiety, daytime hypersomnolence and orthostatic hypotension (Boeve, 2005). Dopaminergic drugs (L-dopa, its analogues and dopamine receptor agonists), are the predominant treatment for PD and can be referred to as dopamine replacement therapy (Rang et al., 2003). They are administered to alleviate the parkinsonism motor symptoms in PDD and sometimes DLB but come with side effects, especially in instances of prolonged or high dosage. Dyskinesia, troubling involuntary movements which typically present in patients who have been on dopamine replacement therapy such as levodopa for two or more years (Rang et al., 2003), is amongst the most troublesome but other side effects include delusions, hallucinations, hypersomnolence and orthostatic hypertension (Boeve, 2005; Caviness et al., 2011). One of the challenges is balancing levodopa levels to minimise dyskinesias whilst maintaining alleviation of muscle rigidity and hypokinesia (Rang et al., 2003).

Prior to the widespread use of dopamine replacement therapies, muscarinic acetycholine antagonists (eg. benzatropine) were used. These anticholinergic drugs dampen acetycholine release and act to address the imbalance between dopaminergic inhibition and acetycholine excitation of striatal neurons (Rang et al., 2003). Anticholinergics can reduce tremor but are not as effective at treating rigidity and hypokinesia as dopaminergic agents (Rang et al., 2003); furthermore they are associated with a significantly higher rate of adverse neuropsychiatric events (Katzenschlager et al., 2003).

Ehrt et al undertook a comprehensive study into the effects of anticholinergics on PD patients and reported declines in cognition (assessed by MMSE) up to 6.5 times higher than subjects on placebo (Ehrt et al., 2010). The authors suggest this may be due to anticholinergic drugs exacerbating a cortical cholinergic deficit already present in PD patients who are borderline PDD. Additionally

muscarinic receptor activity has been associated with reductions in the three main pathological proteins Aβ, tau (Perry et al., 2003), and α-syn (Leng et al., 2001), all of which are in turn linked to deficits in cognition and so it is plausible that blockade of muscarinic receptors by anticholinergics may exacerbate these pathologies and their effect on cognition (Ehrt et al., 2010).

1.1.5.2 Cholinesterase Inhibitors

The main currently available pharmacological option to treat AD patients are acetylcholinesterase inhibitors (AChEIs); aimed at reversing the decline of the cholinergic system and providing symptomatic relief of cognitive impairment through increasing local acetycholine levels at synapses by inhibiting enzymatic breakdown of acetycholine (Francis et al., 1999). However they do not completely reverse or halt cognitive decline, but merely compensate (Simard and van Reekum, 2004).

Donepezil, rivastigmine and galantamine are the three AChEIs currently in use for moderate AD. Clinical trials have shown all three to confer a moderate cognitive improvement to patients; a mean increase of 2.7 on the cognitive section of the Alzheimer's Disease Assessment Scale (Rodda and Carter, 2012). There is argument concerning the clinical relevance of so small a change but the counter argument that 'reduced worsening than would be expected if the patient was untreated' has weight. Currently only donepezil is licensed for use in all stages of AD in the USA but mild to moderately severe in the UK (Ballard et al., 2011a).

It would seem plausible for AChEIs to be efficacious in DLB and PDD given the considerable deficits in the cortical cholinergic system in both dementias (Perry et al., 1994). Rolinski et al. (2012) undertook an extensive review of clinical trials conducted with AChEIs in PDD and DLB patients using Cochrane methodology. They concluded that there was a benefit to patients cognition, that was more pronounced in PDD than DLB, and that there was an improvement in behavioural disturbances in PDD, detected using NPI, but only when taking rivastigmine (Emre et al., 2004). However, the

likelihood of experiencing an adverse event was increased when taking AChEIs and parkinsonism symptoms such as tremor were more frequent in those taking AChEIs (Rolinski et al., 2012).

In contrast to the rather limited clinical benefits, there is evidence from neuronal cell culture work that AChEIs can be neuroprotective. In particular donepezil, at clinically relevant concentrations, has been shown to preserve rat cortical neurons from oxygen and glucose deprivation damage. However this was probably not mediated by AChE inhibition but possibly through alterations in gene expression (Akasofu et al., 2003). Donepezil and galantamine also confer neuroprotection from glutamate and Aβ induced cytotoxicity; again this was concentration dependent and at a physiological concentration (Francis et al., 2005). It should be noted that these neuroprotective actions were the result of pre-treatment of the cells; it remains to be seen whether AChEIs in human AD can generate the same protective effect in a cellular milieu already undergoing cytotoxic processes modelled *in vitro*.

Addressing this question by considering the clinical evidence presents a challenge; the symptomatic benefits of AChEIs may mask the detection of neuroprotection, even with a 'delayed end point' or 'withdrawal' design of trial. 'Randomised start' trials, in which subjects on placebo transfer to active drug and it can be seen whether they gain immediate alleviation of symptoms and 'catch up' with the initial active drug group, may offer the best means to delineate neuroprotection and symptomatic treatment (Mori et al., 2006). Randomised start trials of donepezil, rivastigmine and galantamine have provided some support for a neuroprotective effect; patients on placebo had reductions in cognition that were not regained when transferred to the AChEI, likely due to the missed period of neuroprotection (Tanji et al., 2010).

A further way to assess neuroprotection of AChEIs is using brain imaging of patients participating in clinical trials. Donepezil can safeguard regional cerebral blood flow in the anterior cingulate gyrus and frontal cortex after a year of treatment (assessed by SPECT) (Nakano et al., 2001). Additionally MRI has revealed, in two separate studies, that donepezil maintains hippocampal volumes in AD

patients (Krishnan et al., 2003; Mori et al., 2006). These imaging findings further endorse a neuroprotective role of AChEIs in addition to their primary function of prolonging the presence of acetylcholine at the synapse.

There are indications that AChEIs may have yet another route to efficacy through interactions with Aβ pathology in AD (Francis et al., 2005). Studies conducted on all the main AChEI compounds have shown an increase in the production of the soluble N-terminal APP fragment produced by alpha-secretase cleavage – the first stage of the so called 'non-amyloidogenic pathway' (Racchi et al., 2005). It is likely that this is a post-translational modification as APP RNA levels were unchanged.

However, the main mechanism of action of AChEIs in AD and other dementias remains the inhibition of acetylcholinesterase leading to increased synaptic concentrations of acetylcholine and evidence of disease modification in any form remains controversial (Fisher, 2012; Francis et al., 2005).

1.1.5.3 NMDA receptor antagonists

Deterioration of glutamatergic systems occurs in AD and as the primary glutamate receptor, the NMDA (*N*-methyl-D-aspartate) receptor formed a natural choice of target for pharmacologic intervention in AD. It has been proposed that NMDA mediated excitotoxicity, caused by excessive localised glutamate release engendering toxic damage and loss of glutamatergic neurons, maybe partially responsible for the observed deficit in glutamatergic pathways and reduction of regional and global glutamate levels in AD (Geerts and Grossberg, 2006).

Memantine is a voltage dependant, moderate affinity, non-competitive antagonist of NMDA receptors (Francis et al., 2012). It is considered to partially block the ion channel but is only able to do so when the receptor has been activated by glutamate binding and is thus already open. In this way the effects of memantine are moderated and it is likely this has avoided many of the side effects, like hallucinations, associated with other earlier NMDA antagonists developed to combat NMDA mediated excitotoxicity associated with stroke (Geerts and Grossberg, 2006; Rogawski and Wenk, 2003).

Aβ has been shown to have a cytotoxic effect on cultured cells and this may be mediated through a disruption to Ca^{2+} balance which in turn causes increased susceptibility of neurons to glutamate induced excitotoxicity (Rogawski and Wenk, 2003). Additionally, studies have revealed Aβ can interact directly with NMDA receptors resulting in inappropriate activation and possibly contributing to the excitotoxic mediated degeneration of glutamatergic neurons (Cowburn et al., 1997). Rogawski and Wenk comment on the interesting possibility that memantine can have additional benefits in AD by preventing this interaction of Aβ and NMDA receptors – this is substantiated by cell culture experiments that showed pretreatment of memantine to inhibit the toxic properties of Aβ (Tremblay et al., 2000).

The interactions of memantine with pathological proteins are not limited to Aβ but extend to tau. Memantine has been shown to inhibit the hyperphosphorylation of tau (Li et al., 2004). Thus in a variety of *in vitro* and *in vivo* systems memantine has been established to be neuroprotective notably against Aβ induced neurotoxicity (reviewed by Rogawski and Wenk, 2003), and may therefore slow the neurodegeneration seen in dementia.

Human trails of memantine in AD patients have established that memantine can be beneficial to patients with moderate to severe AD but not mild AD or in patients who have been administered cholinesterase inhibitors (McShane et al., 2006; Rodda and Carter, 2012). The first randomised double blind placebo controlled trial (RCT) was conducted by Winblad and Poritis (1999) and there have since been two more for moderate to severe AD (Herrmann et al., 2011; Winblad and Poritis, 1999). In light of this, in the UK, memantine is licensed for use in treating only moderate and severe AD. Currently most clinical trials of memantine have not exceeded six months making it tenuous to draw conclusions about memantine's role as a neuroprotective agent in humans. Importantly memantine is well tolerated and may also prevent development of agitation and lead to improvements in other psychiatric symptoms such as delusions and irritability in AD patients (Herrmann et al., 2011; McShane et al., 2006).

There are conflicting reports regarding memantine and psychiatric symptoms in DLB and PDD. Ridha et al (2005) describe three patients who developed delusions and hallucinations after initiating memantine treatment, highlighting the possibility of an increased susceptability to NMDA related glutamate dyshomeostasis in DLB and PDD patients when compared to AD patients(Herrmann et al., 2011; Ridha et al., 2005). In contrast to this an RCT found no evidence of psychiatric disturbances in a study population of 72 DLB and PDD patients and at the same time demonstrated a moderate benefit to patients using clinical global impression of change and an improvement in cognition according to MMSE scores (Aarsland et al., 2009). Despite this encouraging outcome the authors stress the need for further studies of memantine in DLB and PDD with larger populations.

Finally there exists the possibility of using memantine alongside AChEIs in a combination therapy due to the differing modes of action and side effects of these classes of drugs, although clinical trials have shown benefit of this strategy to cognition and behaviour; the focus was on AD rather than LBD (Francis et al., 2012; Howard et al., 2012; Tariot et al., 2004).

1.1.5.4 Treatment of neuropsychiatric symptoms

In addition to therapies aimed at restoring cognition which may have additional benefits there are specific treatment options for the neuropsychiatric symptoms of DLB and PDD. It is worth noting that prior to commencing pharmacological treatment other options such as psychosocial interventions like social interaction, music and pet therapy should be explored. These can be particularly beneficial to patients suffering depression (Aarsland et al., 2005b).

For medication of neuropsychiatric symptoms in AD the atypical antipsychotics risperidone and olanzapine have proven the most efficacious; particularly olanzapine with regards to psychosis (De Deyn et al., 2004) and risperidone for aggression/agitation (Frank et al., 2004). Nevertheless use of these drugs is associated with an increase in the incidence of extrapyramidal symptoms, somnolence and stroke, a decrease in cognition and in DLB and PDD a severe reaction termed neuroleptic sensitivity or syndrome (Aarsland et al., 2005a; McKeith et al., 2005).

Neuroleptic sensitivity can occur in up to 80% of DLB patients given neuroleptic medication and is severe (presenting as rapid acceleration of parkinsonism and an impairment of consciousness) in around 50% of individuals with DLB (McKeith et al., 1992) - a similar frequency of occurrence has been reported in PDD (39%) and PD (27%) (Aarsland et al., 2005a) – leading to neuroleptic sensitivity to be included as a suggestive feature of Lewy body dementias (McKeith et al., 2005).

Psychosis and aggression/agitation are not usually permanent symptoms in patients and so medication must be continually reviewed and altered. Depression can be managed in some patients with serotonergic agents such as setraline and citalopram, which are also well tolerated in patients (Sink et al., 2005). Neuroleptic drugs have also been shown to worsen cognitive decline in patients

with dementia, particularly with regard to persecution. Importantly this decline was not related to the severity of Lewy body pathology (McShane et al., 1997).

Hence with DLB and PDD there arises a paradoxical need to balance the treatment of motor symptoms with treatment of psychiatric symptoms on a case by case basis. DLB and PDD are frequently associated with a high sensitivity to antipsychotics, including life threatening neuroleptic malignant syndrome (Ballard and Howard, 2006; Sink et al., 2005). It is clear that there is a significant need for improved symptomatic pharmacological options as well as a medication that can address the underlying cause of the pathology and clinical presentation.

1.1.5.5 Deep Brain Stimulation

A more experimental, non-pharmacological approach to the motor and non motor symptoms in PD is deep brain stimulation (DBS). It is fairly well established that DBS can be beneficial in ameliorating motor symptoms, both as a compliment and alternative to pharmacological dopamine replacement therapies. However the picture regarding DBS and non-motor symptoms in PD is less clear. There are studies showing that sub-thalamic nucleus DBS can both worsen and improve a variety of symptoms. The evidence concerning DBS and hallucinations is amongst the more positive, one study noting an improvement in hallucinations in 18 patients up to six months after surgery (Fasano et al., 2012).

Often DBS studies are clouded by issues such as post-operative micro lesions, which can cause a temporary worsening of symptoms; and the transient effect of ceasing a patient's dopamine replacement therapy, which again can briefly cause a worsening of symptoms. However DBS holds several important advantages over the various drugs used in PDD and DLB in that it does not exasperate any symptoms or have any significant side effects, nor can it interact or interfere with the actions of other medications (Fasano et al., 2012).

1.1.6 Genetics

1.1.6.1 DLB and PDD

The genetics of Lewy body diseases are complex and overlap with each other and with AD, which is unsurprising given the overlap in symptoms and pathologies (Meeus et al., 2012). Cases of familial PD provided the first genetic evidence for a Lewy body disorder in the form of three point mutations discovered in the α-syn (*SNCA*) gene (Polymeropoulos et al., 1997) (reviewed by Hardy and colleagues (Hardy et al., 2009)). Although only a minority of PD cases are familial it served to put the focus of research on α-syn; a 140 amino acid ubiquitous protein localised to the pre-synaptic terminal (Jakes et al., 1994).

Kurz and colleagues conducted a systematic review into familial PDD and DLB and found reports of 24 families with a history of PD and dementia occurring in the same individuals, most had mutations in the *SNCA* gene, some in the gene encoding Bsyn (another member of the synuclein family) (Kurz et al., 2006). There were less examples of familiar DLB to be found, and a more varied mix of mutations, including APOe3/4 (apolipoprotein E), *SNCA* and Bsyn. Importantly APOe4 has also been identified as a genetic risk factor for sporadic DLB (Pickering-Brown et al., 1994). Fascinatingly, some families in the report had a history of both DLB and PDD; that these two dementias can arise from the same mutation gives weight to the notion they exist on a disease spectrum (Kurz et al., 2006).

It has been established that there is a dose-dependent relationship between the number of copies of the *SNCA* gene and disease progression; families with a duplication of *SNCA* develop idiopathic PD with a slow clinical progression. In contrast, in families with a triplication of *SNCA* the disease develops faster and earlier and is more likely to be accompanied by dementia (Chartier-Harlin et al., 2004; Fuchs et al., 2007). The fact that the age of onset of individuals with a point mutation in SNCA is similar to those with a duplication of the gene points to a 'gain of function' i.e. an increase in α-syn's propensity to aggregate as the mechanism by which these genetic alterations achieve pathogenicity (Hardy et al., 2009).

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are known to be a leading risk factor for sporadic and familial PD, by some estimates as much as 5% of familial and 3% of sporadic cases (Liu et al., 2012). LRRK2 encodes a large protein with multiple GTPase and kinase sites which is expressed throughout the brain, interestingly most of the reported mutations affect these enzymatic sites rather than other protein interacting domains suggesting that this enzymatic activity of the LRRK2 protein is the more important with regard to the disease process (Cookson, 2010).

There have been few comprehensive studies screening for LRRK2 mutations in DLB or PDD but there is a high variability in clinical presentation of patients with LRRK2 mutations and some do have dementia (Ross et al., 2006). That the penetrance of LRRK2 mutations are age dependent suggests the action of modifying factors to disease development (Toft et al., 2005a). Finally, in a study screening 242 individuals with dementia (primarily AD), it was concluded that LRRK2 mutations are not common in AD as none of the known LRRK2 mutations were detected (Toft et al., 2005b).

Gauchers disease, a heritable lysosomal storage disorder characterised by a deficiency of glucocerebrosidase due to a mutation in the glucocerebrosidase (GBA) gene, can be accompanied pathologically by Lewy bodies and clinically by parkinsonism (Campbell and Choy, 2012). Given this, it was not surprising that a relationship was found between mis-sense mutations (which typically reduce enzymatic activity) in the GBA gene and both PD and DLB (Bonifati, 2008; Goker-Alpan et al., 2006). Indeed the occurrence of GBA mutation was far higher in DLB cases (present in 23% of cases) than in PD (present in 4% of cases) in the study by Goker-Alpan and colleagues. It is possible that this mutation in GBA may affect processing of α-syn and thereby increase susceptibility to DLB (Cullen et al., 2011).

Despite this evidence that genetic factors can play a role in the development and progression of DLB and PDD there is clearly a large environmental influence. This was elegantly demonstrated by Wang and colleagues who gathered a cohort of 11 pairs of monozygotic twins, in which one of each pair had autopsy confirmed DLB. There was a very low rate of concordance between twins; in only 1 pair did both develop 'pure' DLB with no AD pathology; and only a further 4 pairs of twins both had dementia of different types. It is interesting to note that even when both twins developed dementia there was generally a prolonged interval of up to 9 years between the onset of dementia in the twins suggesting that even if genetics predispose to dementia, environmental factors retain a profound influence on disease progression (Wang et al., 2009).

1.1.6.2 ***Alzheimer's Disease***

There has been considerably more research undertaken into AD genetics than DLB or PDD; in a similar study and in direct contrast to the DLB twin study undertaken by Wang et al (2009) it was established that there is a strong concordance of 78% for AD amongst monozygotic twins (Bergem et al., 1997). As far back as 1991 mutations in the Aβ precursor protein (APP) were discovered by Goate and colleagues on chromosome 21 in familial AD, and Aβ has not left the centre stage in AD research since then (Goate et al., 1991; Goate and Hardy, 2012). The mechanisms through which Aβ is postulated to cause disease will be covered in the pathology and biochemistry sections.

Two more genes responsible for early onset familial AD have been well characterised in the *PSEN1* and *PSEN2* genes (encoding presenilin 1 and 2 respectively); taken together with APP these mutations only account for between 1 and 5% of AD cases (and presenilin comprises 90% of familial AD mutations) yet the estimated contribution of genetics to the risk of AD in the general population is thought to be around 70% (Ballard et al., 2011b).

The majority of *PSEN* mutations are mis-sense resulting in a loss of function. Studies in mice with a lack of presenilin function have shown presenilin to be important for neurotransmitter release, in

particular glutamate, and so paucity of presenilin function can impact LTP (Saura et al., 2004). It is thought that presenilin 1 is required for correct localisation of NMDA receptors to the synaptic membrane (Ho and Shen, 2011; Saura et al., 2004). Mutations in PSEN genes may affect the production of Aβ in humans (Ballard et al., 2011b), a fact which is supported by observations in PSEN knockout mice showing a lack of Aβ production (Saura et al., 2005).

The first genetic risk factor for sporadic late onset AD identified was the *APOe4* allele. Individuals with increased APOe4 alleles had a sevenfold higher risk of AD and an earlier age of onset (Corder et al., 1993), individuals with APOe2/3 alleles had a markedly later age of onset leading to speculation that APOe2 is a longevity gene (Roses, 2006).

Further genetic risk factors for AD have been found through genome wide association studies (GWAS), in which thousands of samples can be screened for common single-nucleotide polymorphisms (SNPs) but none with the effect size of APOe4 have been found to date (Guerreiro and Hardy, 2011; Moraes et al., 2012). Instead 26 SNPs (review in depth by Moraes et al. and published online in full by the National Human Genome Research Institute http://www.genome.gov/gwastudies; Bethesda, USA) have been found which independently confer a minor increase in risk of around 1.5 to 2 fold. Of these SNPs the three most consistently reproduced are those for the clusterin, phosphatidylinositol binding clathrin assembly protein (PICALM) and complement receptor 1 (CR1) genes (Guerreiro and Hardy, 2011; Moraes et al., 2012). These findings have served to highlight three biochemical process in the etiology of AD; inflammation, endocytosis and lipid metabolism, discussed further in the biochemistry section (Guerreiro and Hardy, 2011).

1.1.7 Pathology

The pathological hallmark of LBD are Lewy bodies, first described by Friederich H. Lewy 100 years ago, and which fall into three categories; brainstem predominant (bLB), limbic or transitional and cortical (cLB) – all primarily composed of the protein alpha synuclein (α-syn). Typically bLB appear first and have a more defined morphology. There is a progression of LB pathology from the brainstem, through the limbic regions culminating in the cortex (Braak et al., 2003). It is unclear whether brainstem or limbic LBs correlate to the degree of dementia but a correlation between dementia and cLBs has been reported (Duda, 2004; Harding and Halliday, 2001). Alongside LBs there are other, related, common pathological features; Lewy neuritis, either axons or dendrites containing high amounts of aggregated α-syn and so called 'pale bodies', thought of as a pre-lewy body.

LBs and Lewy neurites share a similar neuroanatomical distribution throughout most brain regions except the cerebellum, where none have been reported. Why this should be so and what is peculiar to cerebellar neurons remains to be elucidated (O'Brien et al., 2006; Wakabayashi and Takahashi, 1997). This is of interest also in view of recent findings in *C9ORF72* mutant amyotrophic lateral sclerosis with frontotemporal dementia cases where cerebellar pathology is a defining feature (Al-Sarraj et al., 2011). Table 1.1.7 summaries the distribution of LBs throughout the brain in DLB and PDD according to Braak stage (taken from (O'Brien et al., 2006)).

Braak PD Stage	Anatomical distribution of LBs
1	Medullary tegmentum, dorsal motor nucleus of vagus, olfactory bulb
2	Locus ceruleus, caudal raphe, reiticular nucleus of medullary and pontine tegmentum
3	Substantia nigra and basal forebrain
4	Medial temporal cortices, amygdale, CA2 in hippocampus
5	Multimodal association cortices, frontal and temporal lobes
6	Unimodal association cortices and primary cortices

Lewy neurites are found in the hippocampus, cingulate gyrus, entorhinal cortex, amygdale and basal ganglia and are an earlier pathological event than LBs (O'Brien et al., 2006).

Another interesting anomaly regarding LBs is their appearance in genetically defined (Lantos et al., 1994) and sporadic AD cases (Hamilton, 2000). That this LB pathology is not a separate coincidence is supported by the presence of LB pathology in early onset genetic AD cases and in the brains of those with Down's syndrome; indeed, these findings appear to establish a link between Aβ and α-syn pathologies (Ballard et al., 2011b; Simard and van Reekum, 2001). However it should also be remarked that α-syn can generate Aβ pathology; as demonstrated by the intensified pathology resulting in crossing α-syn over-expressing mice with APP transgenics (Masliah et al., 2001b; O'Brien et al., 2006).

In many cases of DLB or PDD there is also an occurrence of pathology regarded as being distinctive to AD, namely amyloid-beta (AB) plaques and neurofibrillary tangles (NFT). This can often lead to difficulties in diagnosis and this combination of pathologies has been referred to as Lewy body variant of AD in earlier studies, although this terminology is now less commonly used (Hansen et al., 1990; McKeith et al., 2004).

The primary AD type-pathologies are abeta positive plaques and tau positive threads and tangles. Abeta pathology is principally located in grey matter and spreads throughout brain regions in a topographical manner depending on how advanced the disease is. This topographical spread begins in the basal regions of the isocortex, followed by the hippocampus and entorhinal cortex, then the striatum, brainstem nuclei, and diencephalic nuclei, culminating in the cerebellum and additional brainstem nuclei (Thal et al., 2002).

Tau pathology likewise spreads in a topographical manner, in the early stages of the disease the entorhinal cortex is affected, followed by the hippocampus and finally the isocortex (Duyckaerts et al., 2009). The amount of AD pathology in DLB and PDD varies but can be as severe as is found in AD (Jellinger and Attems, 2008).

1.1.7.1 Amyloid-beta

Alzheimer's disease, like most neurodegenerative disorders, is defined and diagnosed pathologically by aggregated protein deposits in the brain. In the case of AD these are extracellular amyloid plaques formed principally of amyloid-beta (Aβ), and intracellular neurofibrillary tangles (NFT) composed of tau (Duyckaerts et al., 2009). Aβ has been hypothesised to be central to the pathogenesis of AD since it was first purified from leptomeningeal vasculature extracts (Glenner and Wong, 1984). Evidence corroborating this keystone of AD theory has accumulated (Ballard et al., 2011b); most mutations in familial AD and genetic risk factors for sporadic AD affect Aβ metabolism in some way (Tanzi and Bertram, 2005) and cell culture work has shown Aβ aggregation, particularly as oligomers, to be toxic (Takashima, 2009; Walsh et al., 2002).

Aβ is produced through enzymatic cleavage of a transmembrane glycoprotein amyloid precursor protein (APP); beta-secretase cleaves a cytosolic fragment from APP which is truncated again by gamma-secretase into Aβ and the APP intracellular domain. In a physiological state however, the majority of APP is processed by alpha-secretase in a non-pathological pathway that may even by neuroprotective (Lahiri and Maloney, 2010). It should be noted that there is variability in the amino

acid composition of Aβ produced in this way, with the peptide chain terminating anywhere from amino acid 39 to 42 (Duyckaerts et al., 2009).

A similar multifariousness can be found in the descriptions and types of Aβ deposits or plaques. Before the advent of Aβ specific antibodies and immunohistochemical techniques stains such as congo red, thioflavin S and Bielschowski's silver stain were used to visualise plaques and so gave rise to different classifications of plaques. Plaques broadly fall into three categories based on morphology; diffuse, focal or stellate, and are often accompanied by a corona, coined 'neuritic' plaques (Duyckaerts et al., 2009).

Stellate plaques are believed to be astrocytic in origin but are poorly characterised, diffuse plaques, as the name suggests, are variable in morphology across different brain regions (Duyckaerts et al., 2009). That they are commonly found in aged but cognitively intact individuals lends credence to the theory that diffuse plaques are not the primary drivers of neurotoxicity (Dickson et al., 1992).

Focal (or neuritic) plaques were considered the cytotoxic culprits – although current evidence points towards oligomeric species rather than plaques as initiators of toxicity (Walsh et al., 2002). Such plaques are dense, with a core consisting primarily of Aβ-42 (Güntert et al., 2006). It has been estimated that the quantity of Aβ protein within a plaque can be in the region of 100 fmole (Rüfenacht et al., 2005). Neuritic plaques are frequently associated with microglia and NFTs reinforcing the link between Aβ and tau and highlighting the role of inflammation in AD pathology, both discussed later (Duyckaerts et al., 2009).

Aβ can also be found accumulated in the walls of cerebral blood vessels, a phenomenon known as cerebral amyloid angiopathy (CAA). It is likely that ischaemia and microhaemorrhages, attributed to CAA induced damage to vessel walls, contribute to cognitive decline. CAA can arise sporadically in non-demented elderly as well as in association with AD, particularly familial AD (Revesz et al., 2009). In a study by Attems and colleagues it was reported that the severity of CAA and AD pathology were

significantly related; although around a quarter of AD cases examined did not have CAA and a similar fraction of control cases did have CAA (Attems et al., 2005). Recently, BrainNet Europe Consortium multicenter study emphasized the need to unify assessment of vacular alterations including amyloid angiopathy in post-mortem studies (Alafuzoff et al., 2012).

Diagnosis of Alzheimer's disease is based on an assessment of pathology at post mortem. Historically an array of staining techniques were used to visualise this pathology; to achieve consistency between centres, a landmark comparison of staining techniques and assessment criteria across the BrainNet Europe centres was conducted and concluded that use of antibodies against Aβ and hyperphosphorylated tau gave the highest reliability (Alafuzoff et al., 2012; Alafuzoff et al., 2006). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) published guidelines establishing four stages of diagnosis; normal brain, possible AD, probable AD and definite AD (Mirra et al., 1991). The semi-quantitative nature of the immunohistochemical grading recommended by the CERAD report avoided previous issues of inter-centre and scorer variability, particularly when assessing cases with low plaque counts (Duyckaerts et al., 2009). The BrainNet Europe Consortium has introduced a simple and reliable staging protocol to assess tau pathology which is now widely used by neuropathologists (Alafuzoff et al., 2008).

However the CERAD stages do not necessarily relate to the severity of the clinical presentation and so the National Institute on Aging and Reagan Institute criteria (NIA Reagan criteria) were developed to relate the clinical and pathological aspects and to combine the focus on tangles and threads recommended by the Braak staging with the attention given to plaques under the CERAD guidelines (The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease (1997)).

Newell et al. assessed the diagnostic effectiveness of the NIA Reagan criteria and found a high concordance between clinical AD and a 'high probability of AD' at post-mortem according to the NIA

Reagan criteria; additionally the NIA Reagan criteria proved superior than CERAD at distinguishing DLB from AD (Newell et al., 1999).

There are a number of suggested physiological roles for Aβ including an oft proposed participation in a kinase signalling pathway involving c-Jun N terminal kinase (JNK) (Lahiri and Maloney, 2010). Lahiri and Maloney summarise the many other purported functions of Aβ, ranging from providing anti-oxidative protection from metal ions to cholesterol trafficking. Aβ may also function as a neuromodulator at both nicotinic and NMDA receptors through a pathway revolving around phosphorylation of Akt (Abbott et al., 2008). Akt is a serine/threonine kinase B implicated in neuronal survival and LTP. Recently Soscia and colleagues (2010) have reported Aβ to have an antimicrobial role and suggest Aβ production could be a response to microbial pathogens – an idea which is substantiated by the oft described inflammatory processes occurring in neurodegeneration (Soscia et al., 2010).

Aβ has been shown to be released at the synapse into the interstitial fluid following normal synaptic activity. APP is internalised into synaptic vesicles via the same mechanism as synaptic proteins such as synaptophysin – and this is increased with heightened firing of the synapse – resulting in greater production and release of Aβ (Cirrito et al., 2008). This is supported by a brain imaging study suggesting the brain regions most active throughout life (posterior cortical regions including the cingulated and parietal cortices) are the most susceptible to Aβ pathology (Buckner et al., 2005). Despite these many theories Aβ's precise physiological function(s) remain(s) a mystery.

There is controversy about detection of intracellular Aβ (Duyckaerts et al., 2009). The antibodies used in some studies reporting intracellular Aβ may be detecting epitopes common to APP and Aβ. Despite availability of Aβ specific antibodies there is still much variability in results due to differences in immunochemical techniques; however Aho et al conclusively demonstrate the presence of intracellular Aβ in subjects with AD but not in control subjects (Aho et al., 2010). It has been

speculated that Aβ may accumulate intracellularly prior to development of extracellular pathology and cell loss (Duyckaerts et al., 2009).

The amyloid hypothesis, formulated in 1991 by AD researchers, states that aggregated Aβ leads to NFT formation which in turn causes neuronal death and this progressive process manifests clinically as memory loss and other symptoms associated with dementia (Hardy and Allsop, 1991; Selkoe, 1991). A number of predictions were made regarding Aβ and AD; firstly that other genetic causes of AD would have a connection to Aβ, secondly that Aβ would be toxic to neurons and culminating with the pivotal concept that reducing Aβ would assuage the clinical symptoms of AD (Hardy, 2009). Implicit to the cascade from Aβ via NFT to cell death is the notion that Aβ initiates NFT formation and that NFTs are a closer event to cell death than plaque formation.

Despite the above prediction regarding Aβ toxicity, after the discovery that Aβ was the principal component of the extracellular plaques there was considerable debate as to whether it was inert, neuroprotective or neurotoxic (Hardy, 2009; Tabaton and Gambetti, 2006; Walsh et al., 2002).

A significant hurdle to the toxic plaque theory is that poor correlation of plaques to clinical severity and neuronal loss. Whilst it is possible that extracellular Aβ may have a toxic effect on membranes that can be exerted from a distance or cause a predisposition to the effects of intracellular tau aggregation several factors, such as the lack of any known ‘Aβ only’ neurodegenerative condition when there are ‘tau’ only diseases casts doubt on this (Benilova et al., 2012) yet the cases of a mutation in APP alone being sufficient to give rise to AD reaffirms that Aβ must have a role in the disease.

Another criticism of the amyloid cascade is the presence of often substantial Aβ pathology in non-demented controls (Dickson et al., 1992; Jellinger and Attems, 2012; Knopman et al., 2003). Despite this generally being an observation in a minority of surveyed control cases, it serves to cast further

doubt upon Aβ alone being inherently, substantially and consistently neurotoxic to a degree necessary for the emergence of significant cognitive impairment.

Soluble oligomers of Aβ (sAβ) can be isolated from human post-mortem brain tissue and serve as a better correlate to clinical symptoms than plaques (Tomic et al., 2009). Amongst the most compelling evidence for this is that provided in a study by Lesné et al. using Tg2576 mice expressing human APP. These mice developed worsening memory deficits until six months at which age the degree of memory impairment plateaued until 14 months when it began to decline further. A 56kDa species of soluble Aβ (termed Aβ*56) was found to parallel this pattern up until the later decrease in memory at 14 months but the authors conjecture Aβ plaques (which have accumulated sufficiently to take up the mantle of synaptic degeneration from Aβ*56) to be responsible for this later decline (Lesné et al., 2006).

This correlative evidence was elegantly cemented with a causative approach by administering Aβ*56 to rats. A decline in long-term memory mirroring that in the Tg2576 mice was seen; that this decline was transient suggests Aβ*56 was having a physiological rather than pathological effect (Lesné et al., 2006). However there remains the possibility that sodium dodecyl sulphate (SDS) in the buffer could either have artificially caused the formation of Aβ*56 or be responsible for the toxicity behind the observed memory effects in rats (Benilova et al., 2012; Lesné et al., 2006).

Clusterin (APOj) has been shown to promote formation of sAβ and *in vitro* it has been shown that sAβ disturbs neurotransmission and causes cell death. Two of the APP mutations causing familial AD may predispose to formation of sAβ (Benilova et al., 2012). Furthermore, clusterin has been found to associate specifically with Aβ40 containing plaques in the brains of individuals with AD (Howlett et al., 2013).

Neurons contain a heterogeneous mix of Aβ momomers of different amino acid chain lengths, Aβ 40 being the most common under physiological conditions; this may switch to Aβ 42 in pathological

conditions. Changes in the ratio of Aβ lengths, especially 40/42, may be more important than concentrations of Aβ in driving the switch from equilibrium to a pathological cascade. Likewise there is a considerable variation in the toxic oligomers, both those found biologically and those used in *in vitro* assays. Some of which may not exist biologically or have been created by the purification steps used, in particular SDS-page (Benilova et al., 2012). Benilova et al propose that a dynamic equilibrium exists between Aβ monomer, oligomer and aggregate although dimeric and dodecameric species have received the most attention (Wilcox et al., 2011).

The matter of exactly what constitutes a toxic effect is another hurdle for the toxic sAβ theory, and one that has not been helped by issues with some assays such as possible interference of sAβ with the dye in the mitochondrial MTT toxicity assay (Benilova et al., 2012). However the gathering weight of evidence from different assays makes it increasingly probable that sAβ does cause cell death.

In addition to cell death it is generally accepted that sAβ can exert synaptic effects. Antibodies specific to sAβ species show a dendritic pattern of binding which is increased in AD brain and it has been demonstrated using confocal microscopy that sAβ co-localises with PSD95 (Lacor et al., 2004). The authors put forward the hypothesis that sAβ binding to synapses is a substrate for the impairment of new memory formation observed in individuals at an early stage of AD.

This concept is supported by evidence from a number of different modicums that sAβ not only binds to synapses but has a functional effect; inhibition of long term potentiation (LTP) and depression (LTD) using oligomers derived from human AD brain, APP transgenic mice and cells expressing human APP (Shankar et al., 2008; Wilcox et al., 2011). In the mice this was accompanied by a behavioural effect on memory. However this data is associated with some controversy as other groups have been unable to see a cognitive phenotype when introducing sAβ to mice (Benilova et al., 2012). Interestingly this effect of Aβ on LTP forms the functional basis for a recent experimental

therapeutic approach utilising antibodies to clear the toxic Aβ oligomers interfering with LTP (Klyubin et al., 2005). This is discussed further at the end of the Aβ section.

The mechanism by which sAβ might mediate toxicity or interfere with function at the synapse is unclear although recently it has been suggested that decreased phosphorylation of Akt through a NMDA and α7 nicotinic receptor mediated pathway - as a consequence of chronic Aβ exposure - may explain Aβ generated synaptotoxicity (Abbott et al., 2008).

Lacor and colleagues found synaptic binding of sAβ caused an up-regulation of Arc (activity regulated cytoskeletal) protein (Lacor et al., 2004), a product of a synaptic immediate-early gene, the overexpression of which has been linked to synaptic dysfunction and memory impairment (Guzowski, 2002). Arc has been proposed to act detrimentally on a number of pathways and processes within the synapse affecting spine morphology and receptor trafficking. It is also conceivable that sAβ can affect these processes directly (Lacor et al., 2004).

Thus the nature, role and mechanism of sAβ in AD remains to be elucidated and criticisms abound, particularly as to whether the sometimes subtle synaptic effects can fully account for the severity of neuronal loss encountered in late-stage AD (Hardy, 2009). Ultimately it will be necessary to thoroughly characterise toxic sAβ before it can become a realistic target for interventions such as immuno-clearance (Benilova et al., 2012).

Given the overwhelming evidence for Aβ playing a key role in AD pathogenesis it was a natural assumption, and in concord with the amyloid cascade hypothesis, that reduction or complete elimination of Aβ plaques and pathology from the brain would ameliorate the clinical syndrome (Hardy, 2009; Marchesi, 2012). However there has been no success in any of the clinical trials aimed at reducing Aβ levels. There are two general positions taken as to why this may be; the first is that Aβ is the wrong target and possibly another aspect of the disease such as tau or metal ions should be investigated, the second is that the design of the trials was flawed in testing new therapies in

advanced AD patients in which neuronal loss and synaptic damage may be too great for a cessation of the pathological mechanisms of Aβ to have any meaningful or detectable benefit to the patient.

Instead there is an argument for patients with MCI (mild cognitive impairment) to be used in clinical trials. This would require the use of biomarkers to confirm MCI status and assumes that MCI patients will progress to AD, however MCI patients may represent a better hope for halting the neurodegeneration at a less advanced stage and circumvent the issue of the early onset of pathology prior to development of symptoms (Marchesi, 2012). An additional approach has been the use of populations of with a more genetically homogeneous presentation of dementia such as the dominantly inherited Alzheimer's disease network (DIAN) cohort – with the additional benefit of the extensive clinical and pathological characterisation undergone by participants (Morris et al., 2012).

The strategies employed by the therapeutic agents assessed in the clinical trials to date centre around either blocking production of Aβ, increasing its metabolism or clearing Aβ from the brain using antibodies directed against it (Marchesi, 2012). The most (or possibly the only) success has been in immunisation based trials. The concept was successfully demonstrated in transgenic mice over-expressing APP, in which it was possible to remove almost all Aβ deposits in aged mice as well as prevent development of deposits in young mice (Schenk et al., 1999). Of the few individuals who participated in human trials and have had an autopsy Aβ was reduced or absent yet there was no clear clinical improvement in the trials . Additionally a number of important questions remain, for example, there was a reduction brain volume in immunised individuals, also the mechanism by which antibodies clear Aβ remains unclear, the possibilities include stimulating phacocytosis, sequestering of Aβ elsewhere in the brain periphery or simply blocking aggregation (Delrieu et al., 2012). In the Aβ42 immunisation trial published by Holmes and colleagues (Holmes et al., 2008) it is suggested immunisation may have increased production of toxic oligomeric and soluble Aβ species as a direct consequence of the breakdown of plaques (Boche et al., 2010) or by exacerbating the immune response such that further neurodegeneration occurred – indeed this last point was further

suggested by follow-up examination of microglial activity in immunised patients in which it was found that Aβ42 immunisation altered microglial activity (Zotova et al., 2011).

1.1.7.2 Alpha Synuclein

Alpha synuclein (α-syn) is an abundant member of the synuclein family of proteins, of which beta and gamma synuclein are other prominent members. It was first identified as a component of LBs by Spillantini and colleagues using immunohistochemistry (Spillantini et al., 1997). α-syn's structure is intrinsically disordered (i.e. only a primary peptide structure) unless bound to membranes when it takes on an alpha-helical structure and when aggregated in fibrils it assumes a beta-sheet conformation (Auluck et al., 2010). α-syn contains 140 amino acids and is located in the presynaptic terminal where it is thought to have a role in SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors) complex assembly (Burre et al., 2010). Specifically Burre et al demonstrated that α-syn drives formation of the SNARE complex through a chaperone-like activity involving binding to phospholipids and synaptobrevin-2. The authors suggest α-syn provides a proof-reading facility to SNARE complex function that is important with increased synaptic activity and age dependent, a factor which may go part of the way to explaining the vulnerability of aged neurons to a redistribution of α-syn from the synaptic terminal to form LBs.

This is in contrast to previous work summarised by Goedert (Goedert, 2001) that proposes that it is not a loss of function of α-syn that accounts for its role in neurodegeneration, an opinion based on studies showing that inactivation of the SNCA gene in mice does not cause an overt neurodegenerative phenotype (Abeliovich et al., 2000). Additionally as previously mentioned, people with multiple copies of SNCA genes develop PD early and more severely, again suggesting that it is a toxic gain of function responsible for α-syn's pathogenicity (Singleton et al., 2003).

Ultimately there may well exist a level of redundancy or an ability to compensate for a loss of α-syn in its physiological role. Mice lacking the genes for α-syn and gamma synuclein showed a similar

pattern of altered gene expression suggesting gamma synuclein to be involved in some of the same pathways and functions as α-syn and so may contribute to this compensation (Kuhn et al., 2007).

However, it remains α-syn's propensity to aggregate that sets it apart from other synaptic associated proteins. It is believed that α-syn oligomerisation, followed by its aggregation, directly precedes the formation of Lewy bodies and is in some way cytotoxic. α-syn shares a considerable degree of its amino acid sequence with Bsyn which focuses attention on the differences in the sequences to answer the question of why Bsyn does not aggregate. α-syn contains a central hydrophobic, non-amyloid, sequence, made conspicuous by its absence in Bsyn and which when deleted or the hydrophobicity is altered, seems to prevent aggregation and the consequential deleterious effect to neurons, albeit in a fly model (Auluck et al., 2010; Periquet et al., 2007; Vekrellis et al., 2011).

Some of the mutations in the SNCA gene known to give rise to α-syn disorders exert their pathogenic effect through alterations in the amino acid sequence of α-syn which lead to changes in aggregation or oligomerisation. For example the A53T mutation triples the size of the hydrophobic, aggregation driving domain of α-syn to 30 amino acids whilst the A30P and E46K mutations decrease α-syn's affinity for phospholipid binding which promotes dimerisation (Auluck et al., 2010). Furthermore both of these mutated forms of α-syn can affect membrane curvature – an important property in determining the efficiency and frequency of vesicle binding at synapses. Essentially the higher the curvature, the greater the structural strain on the membrane and thus the propensity to bind is higher. A30P and E46K α-syn reduce membrane curvature and so, by this mechanism, may behave as a brake on vesicle binding and neurotransmitter release and cause an accumulation of vesicles in synaptic terminal reserve pool (Auluck et al., 2010; Perlmutter et al., 2009).

The toxicity of α-syn, in sporadic and familial disease, has been proposed to arise in several ways; through inhibition of histone acetylation by α-syn, perforation of membranes and the consequent disruption of ionic balance and finally neuronal death via the inhibition of a neuronal survival factor MEF2D (Beyer et al., 2009a). Beyer et al suggest that in fact there maybe multiple pathways

involving α-syn and other proteins linked to LBs that culminate in the same end-stage pathology of Lewy bodies and neurites.

Another interesting anomaly in the evidence for α-syn as a toxic agent is the survival and apparent health of some neurons that contain aggregated α-syn and that LBs can be found in non-demented individuals at autopsy (Brown, 2010). It was partly in response to this conundrum that different species of α-syn were identified and theories emerged that it may be different forms of α-syn to the aggregates seen in LBs that are driving the cell death and synaptic degeneration.

There is a variety of oligomeric α-syn species reported in the literature and considerable debate as to how they may form; Brown opines that 'molecular crowding' is the most likely although there is also evidence for a prion like seeding mechanism, discussed later. Many of the oligomeric species identified have a stellate, pore like structure and this has been thought to attribute for the formation of pores in cell membranes and increased ion permeability (Brown, 2010).

There is a theory that oligomeric α-syn may assist in forming a link between the reported metal ion abnormalities in DLB and PDD and the biochemical pathology and that increases in local copper ion availability may facilitate toxicity of oligomers (Wright et al., 2009). This is explored further in the section on zinc and other metals in the CNS.

Many synucleinopthies, in particular PD, are characterised by a topographical spread of pathology, which begins in the medulla oblongata and olfactory bulb, spreading to the midbrain/substantia nigra and culminating in cortical pathology, which was first described by Braak and colleagues and now forms the basis for a formal neuropathological staging (Braak et al., 2003).

However the Braak Lewy body staging (Braak et al., 2003) has come under increasing criticism, initially as a consequence of a number of PD cases in which the topographical spread of Lewy pathology did not follow the caudo-rostral pattern used as the basis for the Braak staging. This was

observed in a large number of cases in two separate investigations (Attems and Jellinger, 2008; Kalaitzakis et al., 2008). Furthermore these studies brought the supposed origin of pathology in the medulla – specifically the dorsal motor nucleus of vagus – under question as in a subset of cases there was an absence of pathology in this nucleus. Additionally some cases of preclinical PD (or incidental Lewy body disease) showed development of pathology simultaneously across multiple brain regions which casts further doubt on the applicability of the original Braak staging to all cases with Lewy pathology (Dickson et al., 2008).

In defence of these criticisms Braak and colleagues revised the original 2006 staging criteria in the form of a 'dual hit' hypothesis in which they propose pathology can arise in the olfactory bulb or enteric nerve plexuses of the foregut and that this pathology is initiated by as yet unknown pathogens (Hawkes et al., 2009). The BrainNet Europe Consortium, similar to the approach applied for tau and Abeta, made recommendations regarding the immunohistochemical protocol and assessment criteria (Alafuzoff et al., 2009).

Another pitfall of the 2003 Braak staging is the relationship between pathology and disease/symptom severity. Inherent in the topographic staging is the concept that with increasing spread and density of pathology there is a worsening of disease severity and a progression from motor symptoms to cognitive symptoms (Jellinger, 2008). This is generally accepted to be the case, in particular when dementia is already present there is often a good correlation between pathology and disease severity (Jellinger, 2008; Kövari et al., 2003; Mattila et al., 2000; van den Berge et al., 2012), yet whilst this is found to be true for PDD, this correlation has not been replicated in DLB (Marui et al., 2002). Despite this difference in the relationship between cortical Lewy pathology and cognitive decline in PDD and DLB, cortical Lewy pathology cannot be used to distinguish PDD from DLB (Harding and Halliday, 2001), it is likely that AD pathology may play a significant role in determining disease progression in DLB (Aarsland et al., 2004), in fact Weisman and colleagues go as

far as to suggest that the presence of AD pathology determines diagnostic success in that when AD pathology was high it became much harder to accurately distinguish DLB (Weisman et al., 2007).

Compounding the difficulties in establishing clarity with regards to pathological and clinical severity are reports of cases of cognitively intact individuals with high levels of cortical Lewy pathology and *vice versa* (low/absent pathology in apparently demented patients). Colosimo et al. report multiple cases of clinical PD patients without dementia who, upon autopsy, were found to have extensive neocortical Lewy pathology consistent with that typically encountered in DLB (Colosimo et al., 2003). Furthermore cortical Lewy pathology can be encountered in individuals with no Parkinsonism or dementia (Jellinger, 2004; Parkkinen et al., 2005). In contrast Aho and colleagues found 53% of cases in a cohort of 178 aged brains from individuals with and without dementia had considerable α-syn pathology yet no cognitive decline or dementia (Aho et al., 2008), a finding repeated by other studies (Parkkinen et al., 2008). It is interesting to note that unlike tau and Aβ pathology, Lewy pathology is not regarded as a normal feature of aged brains and that these reports of cognitively intact aged individuals with Lewy pathology are exceptions - albeit relatively frequent exceptions (Jellinger, 2008).

In view of the discrepancies outlined above, it is clear that there are other factors at work in determining both the pattern of progression of Lewy pathology and how this affects the development of symptoms; probably glial and neuronal loss and other pathologies such as tau and Aβ play an important role (Jellinger, 2008; Parkkinen et al., 2008).

Prion disease is characterised by the spread of a pathologically misfolded prion protein which has the unique ability to induce similar misfolding in non-misfolded native prion proteins and thus propagates infection across neurons – probably via trans-synaptic transport (Jucker and Walker, 2011). Prion disease can be spontaneous with no clear cause, genetic in nature or transmissible. There are a number of striking similarities between prion disease and several neurodegenerative diseases such as AD and the synucleinopathies. Firstly both feature misfolded native protein which

forms beta-sheet rich aggregates within neurons, which appear to be toxic to the neurons. Additionally, this pathology spreads in a stepwise manner through the brain rather than arising independently in multiple regions (Alberts et al., 2002; Braak et al., 2003; Jucker and Walker, 2011).

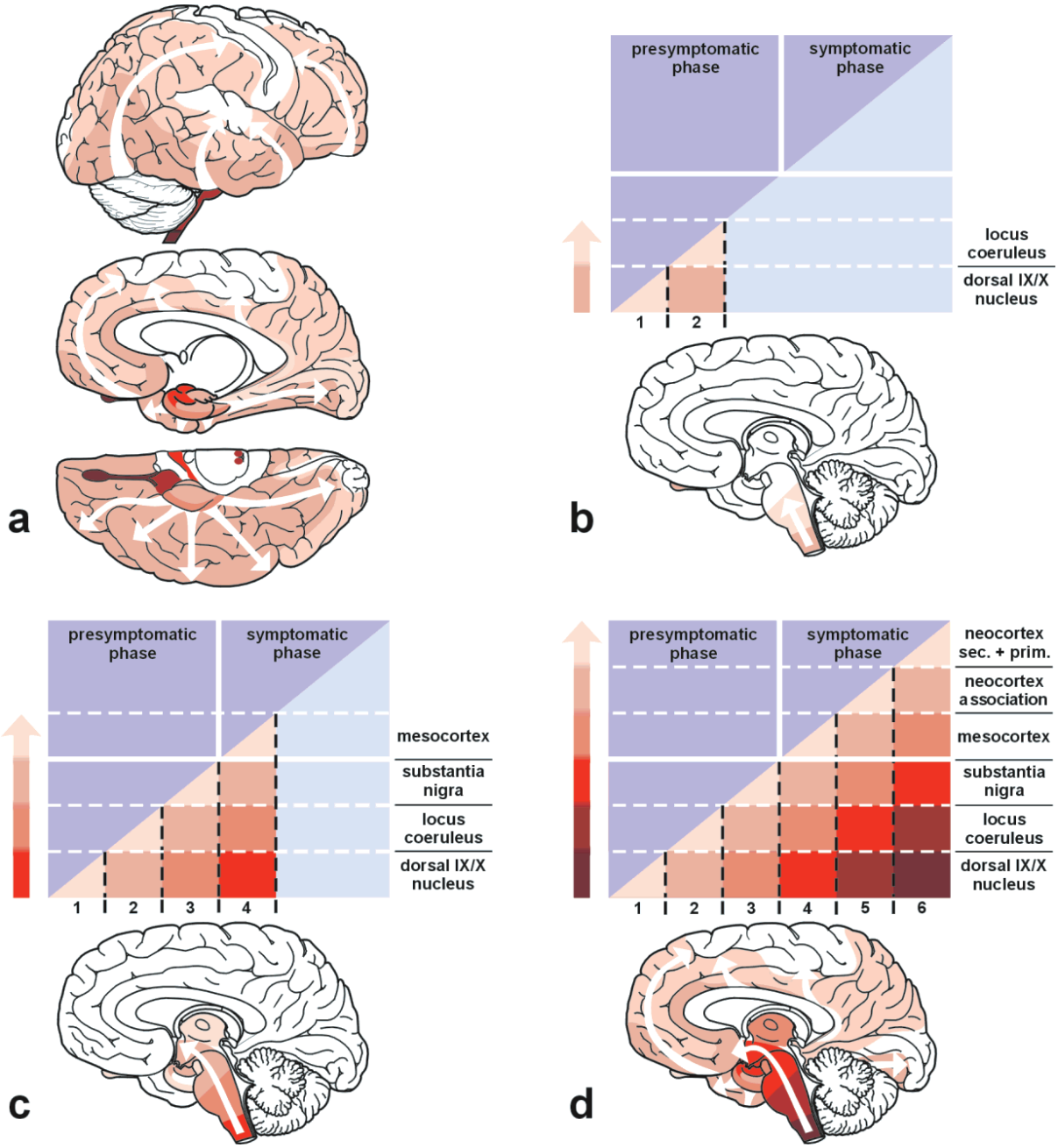


Figure 1.1; Diagrams showing the progression of pathology through the brain in accordance with the different Braak Stages. Taken from Braak et al. 2002. (Braak et al., 2002)

Some of the most compelling evidence for neurodegenerative proteins like α-syn acting in a prion like manner comes from studies showing transmissibility of aggregated proteins, for example introduction of aggregated Aβ to the brains of APP transgenic mice instigated deposition of Aβ in these mice (Kane et al., 2000). Furthermore *in vitro* work on α-syn transmission has clearly shown that α-syn can spread from neuron to neuron (and to glial cells) via endocytosis and that this transmitted α-syn forms inclusions and initiates apoptosis (Desplats et al., 2009; Jucker and Walker, 2011). It has also been known for some time that transplanted cells in PD patients develop α-syn pathology. This is particularly unusual because the grafted neurons are typically between 11 to 22 years old by the time the recipient reaches post mortem and so much younger than most non-grafted neurons with reported α-syn pathology. Additionally grafted neurons in Huntington's disease have not been observed to develop α-syn pathology, casting doubt on this pathology arising as a result of the grafting process (Hansen and Li, 2012). Although Jucker and Walker argue that this is not such compelling evidence for a prion like mechanism as there remains the possibility this α-syn aggregation was independent of the host (Jucker and Walker, 2011).

Kramer and Schulz-Schaeffer (Kramer and Schulz-Schaeffer, 2007) reported accumulation of small aggregates of α-syn at the synapses of DLB post mortem brains that were proposed to interfere with synaptic function (Figure 1). The authors also suggest this is a direct precursor to the development of LBs, and possibly represents a final cytoprotective attempt before cell death. Thus it could be hypothesised that by the time LB formation begins in a neuron it is likely to be too late for continued cell viability and thus focus on the events occurring at the synapse far in advance of LB formation would be of greater potential therapeutic benefit.

Other proteins have been shown to be associated with LBs. A recent proteomic analysis showed there are around 300 different proteins in LBs, of which 34% were intracellular signalling proteins and 19% part of the cytoskeletal network (Leverenz et al., 2007). Interestingly few of the 300

proteins were involved in neurotransmission or synaptic activity so clearly dysfunction in many aspects of cell function and regulation contribute to the pathogenesis and clinical presentation of DLB and PDD.

1.1.7.3 Tau

Hyperphosphorylated tau is the major protein constituent of one of the pathological hallmarks of AD, neurofibrillary tangles (NFTs), the other main molecular component is ubiquitin (in about 60% of NFTs) (Duyckaerts et al., 2009; Kosik et al., 1986; Wolozin et al., 1986). Ubiquitin is a highly abundant protein used as a molecular tag for other proteins requiring degradation by the ubiquitin-proteosome system, this explains ubiquitins presence in such quantities in NFTs as much of the aggregated tau will have been targeted to the ubiquitin-proteosome system in an attempt at removal (Lee et al., 2013). NFTs are intracellular, fibrillar and correlate with cell death and severity of clinical symptoms (Arriagada et al., 1992; Takashima, 2009). Tau is an abundant microtubule associated protein found in the soma and axons of all neurons where it is thought to stabilise the cytoskeleton (Lee et al., 1989). Neurons are inherently vulnerable to defects in intracellular protein trafficking due to the distances of synapses from the cell bodies and the high metabolism and turnover of proteins; hence the notion that NFTs may interfere with (or a lack of physiological tau may inhibit) this delicate and vital process has been gaining prominence (Santacruz et al., 2005). It is delineating the pathways that lead to this compromised axonal transport that is the challenge (Ballatore et al., 2007).

Tau pathology can be subdivided into tangles (aggregation of tau in the cell body), threads (aggregation of tau in dendrites) and dystrophic neurites - the tau component within the corona of neuritic plaques which is axonal in origin (Duyckaerts et al., 2009). In tauopathies tau pathology is commonly encountered throughout the neocortex, in particular layers 3 and 5, the pyramidal cells of the hippocampus, the entorhinal cortex and the olfactory system (Duyckaerts et al., 2009). This topographical progression of tau pathology was formalised into a scoring system to aid neuropathological descriptions and diagnosis by Braak and colleagues in 1991 (Braak and Braak, 1991).

Pre-tangles represent an immature form of tangle composed of hyperphosphorylated tau fibrils which have yet to form NFTs (Bancher et al., 1989) and on the other side of the spectrum are so called 'ghost tangles', the remnants of intracellular NFTs where the neuron has disappeared (Ikeda et al., 1992). This persistence of tau pathology is in contrast to α-syn pathology which does not remain after cell death (see α-syn section for discussion) (Greffard et al., 2010). Consequently tau pathology accumulates in the brain throughout the duration of the disease; this is one of the factors allowing the correlation between tau pathology and clinical severity to be detected (Arriagada et al., 1992).

Duyckaerts et al reported an interesting case of a patient with surgically acquired damage to a section of frontal cortex resulting in the severance of this piece of cortex from the surrounding brain tissue. Upon autopsy Aβ and tau pathology were found throughout the cortex but in the disconnected piece of cortex there was Aβ pathology but an absence of tau pathology. The disconnected area of cortex had a slightly higher neuronal count than surrounding areas ruling out the possibility that impaired neuronal health, due to the severance, was responsible for the lack of tau pathology. The authors hypothesised that the tau pathology spreads through anatomical connections between neurons, almost in a prion like manner, and that it has a degree of independence from Aβ pathology (Duyckaerts et al., 1997).

Indeed, the link between Aβ and tau pathologies remains one of the significant outstanding questions of AD research. Initially, based on observations that APP mutations causing an increase in Aβ production initiated a pathway terminating in production of tau pathology, it was assumed that Aβ pathology was an upstream driver of tau pathology (Goate et al., 1991; Goate and Hardy, 2012); whereas development of tau pathology through mutations was not sufficient to initiate Aβ pathology (as evinced from familial cases of FTD) (Hutton et al., 1998) thus advocating tau pathology as downstream of Aβ (Duyckaerts et al., 2009). However, for sporadic disease the situation may be different as suggested by analysis by Braak et al of a large group of general autopsy cases (without

regard for diagnosis) in which tau pathology was found to be more common than Aβ, and although there are limitations in extending this finding to the general population it remains unexpected (Braak and Braak, 1997; Duyckaerts and Hauw, 1997).

Observations from the primary animal model for AD, APP transgenic mice, add to the disconnect between Aβ and tau pathologies as these mice, despite expressing high levels of Aβ pathology, do not develop tau pathology unless human mutant tau transgene is included (Oddo et al., 2003). Yet conversely tau hyperphosphorylation does occur in APP transgenic mice and in tau transgenic mice there is no Aβ pathology, so perhaps it is not possible to deduce the relationship between tau and Aβ pathology from mouse models (Ittner and Gotz, 2011).

Recently a tau/APP mouse model has been developed, which develops both tau and abeta pathology. It was found that these mice had more severe NFT pathology, hippocampal neuron loss and motor deficits than tau-only mice, highlighting the importance of abeta to tau misprocessing (Héraud et al., 2013). A final point on this is speculation from Ittner and Gotz (2011) that tau deposition may be initiated non-specifically by amyloidogenic proteins, based on insights from research into the rare familial British and Danish dementias in which Bri peptide (the pathological hallmark of these dementias) when injected into tau transgenic mice, aggravates tau pathology in a similar manner to injection of synthetic Aβ.

The six different isoforms of tau derived from the *MAPT* gene differ in binding properties to beta-III-tubulin, the primary cytoskeletal partner of tau (although it should be noted there are other possible candidates for tau interactions from as diverse a range as presenilin1 and cell membranes). The 3R and 4R isoforms (named for the number of Btub binding site repeats) are the most common and exist in a 1 to 1 ratio under physiological conditions (Hong et al., 1998). It is possible alterations in this ratio (maybe due to mutations in the *MAPT* gene) can have pathological implications. Significantly mutations in the *MAPT* gene are known to be sufficient to give rise to neurodegeneration without other pathologies such as Aβ or α-syn, this was proven when *MAPT*

mutations were demonstrated to cause frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP17) (Ballatore et al., 2007; Hong et al., 1998).

Phosphorylation and dephosphorylation of tau by specific kinases and phosphatases controls a dynamic equilibrium of tau binding and detachment from microtubules (MTs) (Drechsel et al., 1992). This equilibrium becomes disturbed during neurodegeneration leading to almost total phosphorylation of all available sites; leading to a dramatic increase in the propensity of tau to form aggregates (Kuret et al., 2005). An important step in the pathway to aggregation are straight and paired helical filaments – beta-sheet enriched forms of tau, which, when formed, can further self-assemble into NFTs (Ballatore et al., 2007).

The question of gain of function versus loss of function with regards to tau toxicity is clouded. Stabilisation of MTs by the compound paclitaxel succinctly demonstrated that pharmacological replacement of absent tau could reverse the deficits in axonal transport arising from a lack of tau-stabilisation of MTs and reduced the motor symptoms observed in the mice (Zhang et al., 2005), thus supporting a toxic loss of function. However the fact that NFTs (and incidentally not Aβ plaques) correlate to clinical severity in humans would suggest that the NFTs and a toxic gain of function cause the neurodegeneration and synaptic dysfunction that results in the clinical symptoms (Arriagada et al., 1992; Ballatore et al., 2007). It is of course possible that the absence of tau in its role as a stabiliser of MTs and the consequent support to axonal transport combines with the physical obstruction to the already impaired axonal transport caused by NFTs thus delivering a dual attack on neuronal integrity.

Whatever the mechanism by which tau exerts its neurotoxicity, it is clear that this toxicity occurs well before the presence of NFTs. Some of this knowledge has come through observations in a tauopathy mouse model showing that hippocampal synaptic dysfunction and microgliosis precede deposition of NFTs (Yoshiyama et al., 2007). There was a marked decrease in synaptophysin positive synapses, followed by impairment of synaptic function, months before tau deposition was evident.

Furthermore immunosuppression reduced the damage to hippocampal neurons and improved survival of the mice, highlighting the role of inflammation and microglial induced damage to neurons.

In addition to tubulin, tau has proven binding and interactions with dynamin, a tyrosine kinase protein called FYN and PSD95. Phosphorylation of tau increases its binding to FYN which propels FYN to the dendritic spines where FYN plays a crucial role in mediating the interaction of NMDA receptors and PSD95 through phosphorylation of NMDA receptors (Ittner and Gotz, 2011). This pathway is thought to be instrumental in Aβ induced excitotoxicity based upon studies that have demonstrated tau knockout mice to be less susceptible to Aβ and experimentally induced seizures (Ittner et al., 2010; Roberson et al., 2007).

1.1.7.4 Mixed Pathologies

DLB, PDD and AD are often thought of as possessing distinct pathologies instrumental in defining the diseases from one another yet the reality is far more complex. Amyloid and tau pathology can occur in DLB and PDD and *vice versa* for α-syn pathology in AD (Jellinger and Attems, 2008). In an assessment of the prevalence of typical AD pathology in PDD, DLB and PD, Jellinger and Attems found PDD to be associated with higher levels of Aβ pathology than PD (around two-thirds of PDD patients had severe Aβ loads). The DLB cases (although less than half the number of PDD at 20) were characterised by higher quantities of α-syn and Aβ pathology. AD pathology was a greater determinant of cognitive decline than LB pathology in PDD cases and in both PDD and DLB cases where AD pathology was severe, cognitive scores were lowest, highlighting the importance of AD pathology to the clinical syndrome of DLB/PDD (Jellinger and Attems, 2008). The relationship between different pathologies remains intricate but it appears clear that a combination of pathologies is a better predictor of severity of the clinical aspects of the disease than severity of any one type of pathology (Compta et al., 2011).

Current guidelines for pathological diagnosis, according to McKeith et al, are that a higher severity of DLB pathology and a lower severity of AD pathology increase the probability that the observed pathology explains the clinical syndrome of DLB (McKeith et al., 2005).

1.1.8 Mild Cognitive Impairment (MCI)

The concept of MCI was developed in response to the increase in patients presenting at memory clinics that had an impairment of cognition, generally memory, but did not yet meet the requirements for a diagnosis of dementia. Despite initial assumptions that MCI was merely prodromal dementia it is now accepted that not all MCI cases progress to dementia, often vascular insults or depression can be the underlying cause of MCI, however the majority of MCI cases are amnestic and progress to AD (Mufson et al., 2012).

Pathologically MCI is characterised by a roughly intermediate degree of Aβ deposition (in comparison to controls and AD) although often levels are not significantly different to controls (when assessed at post mortem and by imaging studies) and so it would appear unlikely that Aβ pathology is driving the change from MCI to AD (Mufson et al., 2012). However Aβ oligomers may be of more relevance in this regard as it has been reported that the ratio of Aβ42 to Aβ40 can distinguish AD and MCI from controls. Additionally work in human post mortem tissue found that accumulation of Aβ oligomers (particularly dimers and pentamers) in the frontal cortex was inversely correlated to MMSE score and levels of synaptic proteins, including the post-synaptic marker PSD95, these observations were supported by experiments in APP transgenic mice that showed Aβ oligomers and PSD95 co-precipitated at dendritic sites (Pham et al., 2010). This suggests that Aβ oligomers are initiating a deleterious chain of events at the synapse leading to synaptic deterioration which manifests clinically as an advancing cognitive impairment. Exactly what the sequence of events and proteins connecting Aβ oligomers and PSD95 is remains to be elucidated. Possibly Aβ oligomers cross the synaptic cleft from pre to post synaptic terminal via exocytosis and endocytosis to then affect receptor scaffolding and dendritic spine morphology.

1.2 Biochemistry of dementia

1.2.1 Beta-III-tubulin

Beta-tubulin is a key component of the cytoskeleton, with several isoforms, one of which, beta-III-tubulin, is the solely expressed in neurons (Sullivan, 1988). This has led to widespread use of Btub as a marker for neurons in biochemical and cellular studies.

1.2.2 Synaptic proteins and process

Synaptic dysfunction has been of interest to dementia research since Davies et al (1987) first showed an increased loss of synapses in patients with AD (Davies et al., 1987). Synaptophysin (SPP) has been one of the most popular means to assess both the functional state and relative quantity of synapses in dementia. A number of studies have shown decreases in SPP in human post-mortem tissue, mainly from AD patients (DeKosky et al., 1996; Masliah et al., 1989; Pozueta et al., 2012). The picture is less clear in LBD, one study showed no link between lewy bodies and SPP (Clare et al., 2010; Revuelta et al., 2008); others have shown decreases in synaptic density in LBD compared to controls (Brown et al., 1998) and no significant difference between SPP levels in controls and LBD (Hansen et al., 1998). Probably the choice of brain region and type of LBD cases selected have some impact on the variability of results.

1.2.2.1 Drebrin

Drebrin is an actin binding protein expressed in two isoforms in humans (A and E) (Shirao et al., 1988; Shirao et al., 1990). Drebrin A (the adult isoform) is found only in neurons, mainly in dendritic spines, where it promotes extension of the spine through its interaction with actin and the cytoskeleton (Ivanov et al., 2009). A lack of drebrin *in vitro* caused a decrease in spine density and individual spine length. Drebrin A can also influence glutamatergic transmission, altering the balance of excitatory glutamatergic transmission at the expense of inhibitory GABAergic transmission (Han and Kim, 2008; Takahashi et al., 2003).

Drebrin targets post synaptic density scaffold protein (PSD95) and NMDA receptors to the post synaptic membrane (Han and Kim, 2008). Decreases in drebrin have been reported in AD and DLB, the decrease in AD correlated with the severity of dementia and in DLB it was suggested that this decrease was activity dependent due to reduced synaptic transmission caused by the observed presynaptic accumulation of α-syn (Kramer and Schulz-Schaeffer, 2007; Shim and Lubec, 2002). These findings, coupled with the importance of spines for long term potentiation (LTP) – the major cellular correlate of learning and memory – highlight the significance of drebrin and spines to neurodegeneration (Hering and Sheng, 2001).

1.2.2.2 Synaptophysin

Synaptophysin (SPP) is a 38 kDa protein associated with synaptic vesicle membranes. Since its discovery in 1985 (Wiedenmann and Franke, 1985) it has been well established as a biochemical marker of synaptic vesicles and following universal acceptance of the so called 'kiss and run' theory of vesicle endocytosis and recycling of vesicles within synaptic terminals by extension SPP became a marker of the presynaptic terminal itself (Valtorta et al., 2004). SPP being the most abundant synaptic vesicle protein has greatly helped establishment of this role.

SPP is purported to be employed in vesicle trafficking and exo/endocytosis, including interactions with other vesicle associated proteins such as VAMP2, participation in the formation and fusion of a membrane channel to allow rapid neurotransmitter release and a role in clathrin dependent endocytosis (Valtorta et al., 2004).

SPP is encoded by the *SYP* gene and several groups have produced knock-out mice (Eshkind and Leube, 1995; McMahon et al., 1996), initial studies found no overt phenotype suggesting a degree of redundancy in the role of SPP at the synapse or subtlety of the behavioural effect of SPP depletion beyond the capacity of animal models to detect. However, Schmitt et al recently showed that in mice lacking SPP there were learning and memory deficits such as reduced object recognition and Morris water maze performance; thus it would seem SPP is a vital component for normal learning and memory (and thus healthy synaptic function) (Schmitt et al., 2009). The reason for the negative findings in the earlier studies remains unclear.

The delivery of vesicles to the appropriate cell membrane docking site is a complex task requiring a high degree of precision and with the potentially significant consequences of a failure in this it is no surprise that mammalian cells have around 20 different proteins dedicated to this task (Jahn and Fasshauer, 2012). They are referred to as SNARE proteins, divided into transmembrane and vesicle SNAREs, and with specific SNARE proteins for each intracellular compartment. In neurons SNARE

proteins control docking of vesicles to the synaptic membrane immediately prior to release of neurotransmitter and the activity of SNARE proteins are in turn controlled by an assortment of other mediator proteins such as the Rab protein family and GTPases (Alberts et al., 2002; Sollner et al., 1993). α-syn has been implicated in assisting SNARE function under physiological conditions and altering it under pathological conditions (Burre et al., 2010; Garcia-Reitbock et al., 2010).

It has recently been demonstrated that in transgenic mice expressing a truncated, more aggregation-prone, form of α-syn there was a redistribution of SNARE proteins at synapses in the striatum (Garcia-Reitbock et al., 2010). This resulted in decreased vesicle release and dopamine signalling and suggests a toxic gain of function of aggregated α-syn.

Synaptophysin has been consistently found to be decreased in the PFC in AD compared to non-demented control cases (Clare et al., 2010; Davies et al., 1987; Leuba et al., 2008; Minger et al., 2001; Terry et al., 1991). Additionally SPP has been found to be decreased in the hippocampus of AD patients (n=6) and to correlate to cognitive decline and Braak stage, although no significant change in SPP levels was reported in the entorhinal and occipital cortices and the caudate nucleus. There was no change in beta-III-tubulin levels across these regions (Sze et al., 1997).

1.2.2.3 PSD95

PSD95 is a 95kDa post synaptic protein involved in the cellular scaffolding and regulation of membrane expressed neurotransmitter receptors at the post synaptic terminal**.** PSD95 is widely recognised to have roles in synaptic function including regulation of the localisation of membrane proteins and protein trafficking (Han and Kim, 2008). Whilst it is likely that PSD95 is important in directing NMDA receptor localisation to the synapse, there is a degree of redundancy in this role as PSD95 knockout mice show no alterations in NMDA receptor expression or localisation, nor was there any difference in synaptophysin levels between wild type and mutant mice. Taken together with the pronounced alteration in the frequency of LTP-inducing synaptic activity in PSD95 knockouts, the mediation of which is likely undertaken by post synaptic phosphatases and kinases, this suggests that PSD95 is crucial for downstream mediation of NMDA related LTP and does not have an influence on the pre-synaptic terminal (Migaud et al., 1998).

PSD95, a member of the MAGUK (membrane associated guanylate kinases) family of proteins, is the most abundant protein of the PSD (post-synaptic density) and is one of the most extensively characterised proteins of the PDZ domain containing proteins – a protein interaction domain allowing multiple binding to other peptides, common in scaffolding proteins (Kim and Sheng, 2004). PSD95 can form oligomers within the PSD which may facilitate clustering of its interaction partners.

AMPA (α-amino-3hydroxy-5-methyl-4-isoxazole propionate) receptors are ionotropic glutamatergic receptors (Honoré et al., 1982), which undergo an increase in number at the post-synaptic membrane as a focal part of LTP (Shi et al., 1999). Studies from hippocampal slice culture overexpressing PSD95 and rats expressing mutant PSD95 in part of the cortex have shown that PSD95 is elemental in recruiting AMPA receptors to the post-synaptic membrane (Ehrlich and Malinow, 2004). This is in direct conflict with observations made by Migaud et al in the PSD95 knockout mice, in which the frequency and amplitude of excitatory post-synaptic currents (EPSCs) were increased in the absence of wild-type PSD95. Ehrlich and Malinow postulate that this

discrepancy is due to the presence of compensatory mechanisms revolving around a truncated form of PSD95 in these knockout mice (Ehrlich and Malinow, 2004; Migaud et al., 1998).

In animal models of AD, PSD95 has been shown to be perturbed by the associated pathology in line with current theories that AD and dementia in general are synaptic failures. Using mouse amyloidopathy and tauopathy models, Shao et al measured an age-related decrease in synaptic PSD95 (which was importantly not explained by neuronal death) in both models and a redistribution of PSD95 to the neuronal cell body (Shao et al., 2011). The same mice develop age-related impairments in spatial memory which were elegantly demonstrated to be associated not with the significant increase in pathology but to the reduction in PSD95 occurring some months after pathology peaks (Oakley et al., 2006).

In the human brain the connection between pathology and PSD95 is markedly less clear. Shao and colleagues found the variability in the levels of PSD95 between cases too large for quantification (Shao et al., 2011). Other studies have reported decreases in PSD95 in temporal cortex of AD cases and in synaptosomes prepared from AD post-mortem tissue (Gylys et al., 2004; Proctor et al., 2010). Proctor and colleagues speculate that the observed reductions in scaffolding proteins such as PSD95 and SAP-102 (synapse-associated protein of 102 kDa – involved in regulation of NMDA receptor activity and expression (Müller et al., 1996)) - may explain the decrease in NMDA receptors and consequent cognitive decline due to impaired LTP and LTD (the opposing weakening of synaptic strength in an activity dependent fashion (Bear et al., 2006)). An alternative explanation is that loss of PSD95 is implicated in NMDA excitotoxicity and synapse loss which manifests as cognitive decline (Proctor et al., 2010).

However Leuba et al, using semi-quantitative immunohistochemical and western blot analysis of PSD95 levels in the hippocampus and prefrontal cortex from a small cohort of six AD brain, found an increase in PSD95 levels compared to non-demented controls. It is unusual for increases to be detected in synaptic proteins in post-mortem neurodegenerative tissue; the authors defend their

findings with the postulation that ubiquitin-proteosome degradation of PSD95 may have been impaired or that aberrant axonal transport, possibly due to tau aggregation, lead to a build up of PSD95 (Leuba et al., 2008). In support of this concept, it has been shown that inhibition of the proteosome prevents PSD95 mediated, NMDA-induced internalisation of AMPA receptors and the resultant LTD (Colledge et al., 2003).

1.2.3 Zinc and other metals in the CNS

Recently the role of metal ions in neurodegeneration has been receiving increased attention, with an emphasis on the ions of the three most biologically abundant 'trace' elements iron, copper and zinc (Roberts et al., 2012) (Note in this section all reference to metals is to the ions of these metals).

The majority of biological Zn is located bound to a class of proteins called metallothioneins, with 'free' Zn comprising a minority of total biological zinc. Metallothioneins play a crucial role in Zn homeostasis by acting as Zn reservoirs and regulating the availability of Zn in its various roles. There are four isoforms of metallothionein in humans (MT-1 to 4), with MT1, MT2 and MT3 all expressed in the CNS, (principally in astrocytes but also in neurons and microglia). Studies in mice with the metallothionein gene deleted have established the importance of this family of proteins for protection from heavy metal toxicity, cognition and oxidative stress (Santos et al., 2012).

Zn, Cu and Fe all cause Aβ to aggregate and Aβ has binding sites for Zn and Cu, and for Fe under acidic conditions (Bush et al., 1994). Importantly the affinity of Aβ for these ions is within the physiological concentrations of synaptically released Zn and Cu (Roberts et al., 2012). In 1991 a clinical trial in which AD patients were orally administered zinc supplements was abandoned after only a few days due to an alarming deterioration in cognition in the AD group, unfortunately no biochemical investigation was undertaken but reports of zinc aggregating Aβ have been suggested as a cause for this cognitive decline (Bush et al., 1994; Kaiser, 1994).

Zn is required for the successful function of alpha secretase thus it could be speculated that the reductions in Zn levels seen in aging and to a greater extent in AD brains may affect activity of alpha secretase and possibly drive APP processing towards the amyloidogenic pathway. However it is likely that Zn is required at relatively low concentrations and binds with high affinity to alpha secretase and so a depletion of local Zn is far more likely to affect other functions first. Likewise Cu is required for beta secretase activity and in the case of Cu it has been demonstrated that reductions in Cu push

the equilibrium of APP processing to the amyloidogenic route (Edwards et al., 2008; Roberts et al., 2012).

Both Zn and Cu are found at high levels within plaques (Lovell et al., 1998), possibly this represents an entrapment of these ions and thus a detraction from their physiological roles, alternatively these ions may remain from the reported binding and aggregation of soluble Aβ (Bush et al., 1994). However, as focus on the toxicity of pathological proteins in neurodegeneration has shifted from the gross aggregates to soluble oligomeric species so has research on metalobiology of neurodegeneration (Roberts et al., 2012).

Zn binds to histidine residues within the Aβ peptide and in this way assists formation of bonds between the same peptide and between other Aβ peptides, thus precipitating aggregation (again this is possible at physiological synaptic concentrations of Zn) (Bush et al., 1994; Curtain et al., 2001). Interestingly, mice and rats have a sufficiently different sequence of amino acids at the Zn, Cu and Fe binding sites to the human peptide that metal binding does not occur, raising the interesting possibility that this explains the unique lack of age related Aβ deposits in these mammals (Roberts et al., 2012).

Cu and Fe have been implicated in oxidative damage; binding of either ion to Aβ can increase production of hydrogen peroxide (a mediator of oxidative damage) and a decrease in availability of Cu drives the Aβ-Cu interaction to membrane lipid rafts, likely amplifying any toxic effect (Hung et al., 2009). It has been speculated that at low concentrations, Zn binding to Aβ may inhibit Cu binding and thus provide protection from Cu-mediated Aβ-induced hydrogen peroxide production and oxidative damage (Cuajungco et al., 2000). An additional postulated neuroprotective effect of Zn is blockage of the membrane pores formed by Aβ – thought to be one of the primary toxic mechanisms of synaptic Aβ oligomers (Jomova et al., 2010). Thus it can be seen the exact role of Zn in AD pathology remains, to some extent, contradictory.

Deductions about the role of Zn and other metals in Lewy body dementias can be made from research into Parkinson's disease metalobiology. Reports that PD can be caused by environmental exposure to heavy metals are conflicting, some reports have epidemiologically linked Zn exposure to PD (Rybicki et al., 1993; Uversky et al., 2001) whilst others have not replicated this finding (Gorell et al., 1999).

It has been established that Zn and Fe are increased in the substantia nigra in PD (Dexter et al., 1989) but the physiological relevance of this remains to be elucidated (Barnham and Bush, 2008). Metal chelation in a mouse model of proteosome-inhibition induced nigro-striatal degeneration (commonly used to simulate aspects of PD) prevented dopaminergic neuron loss and restored motor abilities (Zhu et al., 2007), the authors propose this effect to be caused by removal of excess Fe however Barnham and Bush remark that chelation of Zn is equally possible as an explanation.

Zn has been demonstrated to propagate α-syn aggregation under *in vitro* conditions by a number of groups. Kim et al found Zn to be able to precipitate α-syn and decrease α-syn's ability to perform a chaperone-like activity with damaged proteins (Kim et al., 2000). In another study Zn promoted the formation of SDS resistant α-syn dimrs, an activity that was inhibited by magnesium ions (Golts et al., 2002). Perhaps the most compelling evidence comes from work by Paik and colleagues in which Zn-induced, self-oligomerisation of α-syn, was dependent on a pH of 6.5, ceasing at a pH of 7.5, and in contrast to metal ion induced aggregation of Aβ, which is not pH dependent (Paik et al., 1999). More recently these findings have been corroborated by Yamin and colleagues who demonstrated the fibrillation of oxidised α-syn by Zn, highlighting the importance of oxidative stress to Zn mediated neurotoxicity (Yamin et al., 2003).

However, in a study by Uversky and colleagues, incubation of Zn and α-syn did not produce fibrils after 100 hours (Uversky et al., 2001). Possibly pH, ionic concentration and the species of α-syn influence aggregation, and despite fairly convincing *in vitro* work demonstrating a possible role for Zn in α-syn's pathogenicity, it remains to be shown whether this translates to *in vivo* models.

Zinc is enriched at many glutamatergic synapses, including those in the hippocampus and neocortex, and it is now widely accepted that zinc is co-released with glutamate and other neurotransmitters and thereby acts as a long term modulator of synaptic plasticity (Sensi et al., 2009). The zinc transporter (ZnT) family of proteins remove Zn from the cytoplasm into vesicles or to the extracellular space and the ZIP (Zrt-and-Irt-like) proteins govern cytoplasmic uptake of Zn, see figure 1.2.3 for more details (Gaither and Eide, 2001).

One of the predominant and best characterised post-synaptic binding partners of Zn is the NMDA receptor. Zn has the remarkable ability to inhibit this receptor in a non-competitive voltage independent manner across an extraordinary concentration range (from nanomolar to micromolar) depending upon the subunit composition of the receptor. The NR2A subunit possesses a particularly high affinity for Zn such that even at resting conditions and ambient Zn levels there is inhibition. Thus, it is apparent that Zn acts in a tonic and phasic manner at glutamatergic synapses (Paoletti et al., 1997; Paoletti et al., 2009).

Mutations in the Zn binding site on the murine glycine receptor prevented Zn binding, and resulted in a post-synaptic potential of greatly reduced amplitude; this was accompanied by extreme motor deficits in the animals. Since no alterations in receptor localisation or expression were detected, the observed effect was attributable to the inability of Zn to potentiate the receptor and provided the first evidence for Zn acting as a neuromodulator (Hirzel et al., 2006).

Another recently purported synaptic role for Zn is at the PSD. It is established that a fraction of vesicular Zn enters the postsynaptic terminal via Ca^{2+}/Zn^{2+} ion channels. Baron and colleagues demonstrated this Zn binding to a domain on the SHANK3 protein (a major component of the PSD instrumental in organisation of PSD structure) (Baron et al., 2006) and proposed that the resultant improvement in the ordering and density of the SHANK3 helical fibres condenses the PSD matrix to cluster receptors and enhance neurotransmission; part of this enhancement is likely to involve Zn recruiting further SHANK3 molecules and a consequent enlargement of the PSD through the

increased availability of SHANK3 cytoskeletal and receptor anchoring sites (Gundelfinger et al., 2006). Yet it remains to be proven that this Zn-SHANK3 ordering of the PSD occurs in a cell model.

In addition to interactions with Aβ, Zn has been shown to promote phosphorylation and aggregation of tau. Under physiological conditions Zn concentrations remain low outside of synaptic vesicles but have been reported to rise significantly in the cytosol in AD. Mo and colleagues demonstrated low micromolar Zn to promote formation of tau fibrils (Mo et al., 2009); whilst another study showed elevated Zn concentrations to propel tau phosphorylation and that this was a bimodal relationship as lower Zn concentrations dephosphorylated tau (Boom et al., 2009).

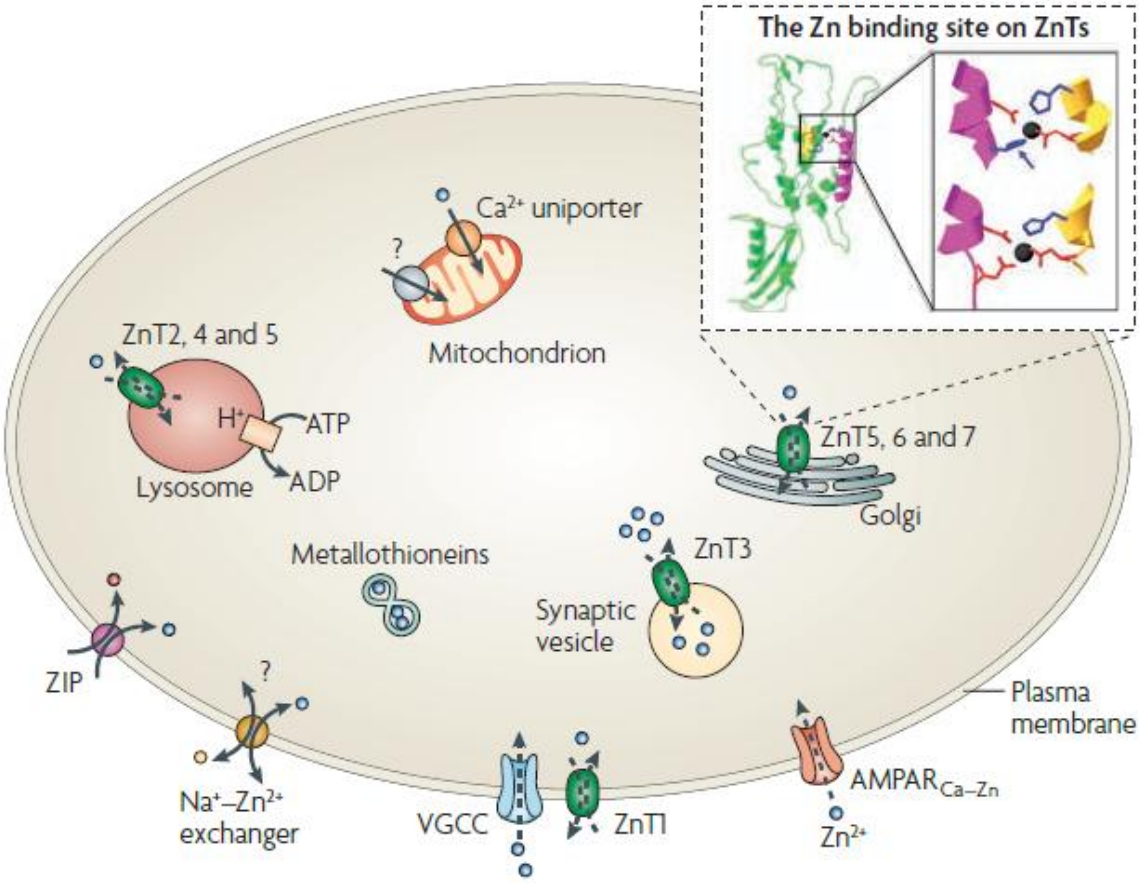


Figure 1.2.3; Pathways and systems regulating Zn^{2+} homeostasis in neurons.

Zn^{2+} enters neurons mainly through activated voltage-gated Ca^{2+} channels (VGCCs) and Ca^{2+}- and Zn^{2+}-permeable GluR2-lacking AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors (AMPARCa–Zns). At the plasma membrane level, Zn^{2+} transporter 1 (ZnT1) controls Zn^{2+} efflux but may also interact with the L-type VGCC that regulates Ca^{2+} and Zn^{2+} influx. The Na^{+}–Zn^{2+}

exchanger, a putative member of the Na^{+}–Ca^{2+} exchanger superfamily, can move Zn^{2+} in or out of neurons depending on the Na^{+} gradient; in addition, Zn^{2+} importing proteins (ZIPs) may act as H^{+} or $HCO3^{-}$ Zn^{2+} co-transporters and facilitate Zn^{2+} influx. Inside of neurons, vesicular ZnTs act as H^{+}–Zn^{2+} exchangers and are located on the Golgi apparatus and other secretory vesicles. Mitochondria also sequester Zn^{2+} through the activation of both the Ca^{2+} uniporter and a Ca^{2+} uniporter-independent mechanism that has not yet been molecularly identified. Finally, metallothioneins greatly contribute to the maintenance of Zn^{2+} homeostasis, as these are the major Zn^{2+} buffering peptides in neurons. The structural model of the Zn^{2+} binding site of a ZnT protein, based on the homology with the bacterial transporter YiiP, shows the striking similarity between the coordinating residues for the Zn^{2+} binding site of ZnTs (top) and the bacterial transporter (bottom). ER, endoplasmic reticulum. The inset is reproduced from (Ohana et al., 2009) whilst the rest of the figure and caption are from (Sensi et al., 2009).Zinc and Depression.

A number of observational studies have linked Zn to depression in humans including several studies that have shown serum Zn levels inversely correlate to the severity of depression (McLoughlin and Hodge, 1990; Nowak and Schlegel-Zawadzka, 1999; Szewczyk et al., 2011). Furthermore administering the anti-depressant therapies citalopram, imipramine, electroconvulsive shock and various combinations of these to rats increased serum Zn levels by up to 30% in the hippocampus, with smaller increases in other regions including the frontal cortex (Nowak and Schlegel-Zawadzka, 1999). This is supported by observations that Zn levels remain low in human patients with treatment resistant depression (Maes et al., 1997), that anti-depressants in humans may raise Zn levels and that Zn administration may have an anti-depressant effect (Levenson, 2006; Szewczyk et al., 2011).

It is well established that the selective serotonin reuptake inhibitors (SSRIs) class of anti-depressant drug exert their anti-depressant effect through blockage of 5-HT (serotonin) reuptake at the synaptic cleft, this causes a chronic down regulation of 5-HT receptors and thus reduces 5-HT neurotransmission and alleviates some of the symptoms of depression (Levenson, 2006). An investigation into the molecular mechanisms by which Zn and SSRIs might interact to achieve the reported enhancements of anti-depressant effect ran contrary to the hypothesis that Zn would magnify the inhibition of 5-HT reuptake, and instead, showed addition of Zn to cortical slices

returned 5-HT reuptake to levels of controls despite presence of one of the three main SSRIs (García-Colunga et al., 2005). It is possible this unexpected action of Zn may be explained by a report that Zn, through allosteric modulation, can act as both an agonist or antagonist at the 5-HT_{1A} receptor (depending on concentration) (Barrondo and Sallés, 2009).

Despite regulation by the blood-brain barrier, Zn levels in the cortex and in particular hippocampus, are susceptible to chronic changes in the dietary intake of Zn (Takeda and Tamano, 2009), thus observations that animal models of dietary Zn restriction caused anxiety and depression-like symptoms suggest a direct role for Zn in mediating the neurochemistry of these conditions (Joshi et al., 2012; Tassabehji et al., 2008; Whittle et al., 2009).

The glutamatergic system has been increasingly implicated in depression, partly as a response to shortcomings in the longstanding hypothesis that the monoamine system mediates depression (inadequacies in treatment response and an unexplained lag between pharmacological actions and emergence of therapeutic benefit), and through observations that NMDA receptor antagonists have an anti-depressant effect and that glutamatergic signalling is increased in depression (Sanacora et al., 2012).

Zn release has been shown to down-regulate glutamatergic signalling through a variety of mechanisms (comprehensively reviewed by Smart and colleagues) including non-competitive antagonism of NMDA receptors in a fashion that is independent from the blocking actions of Mg^{2+}, but sensitive to Ca^{2+} levels, which rise in response to increased glutamatergic activity to form part of the glutamatergic excitotoxic pathway; and stimulation of GABA release from inhibitory interneurons – which decreases glutamate release (Smart et al., 1994). Indeed Joshi et al suggest that Zinc's antagonistic action at NMDA receptors may explain the alleviation of depressive and anxiety like symptoms upon administration of Zn to mice (Joshi et al., 2012). Additionally Zn has been implicated in neurogenesis and increases in BDNF (Szewczyk et al., 2011).

1.2.3.1 ZnT3

At the synapse zinc is sequestered into synaptic vesicles by a synapse-specific member of the zinc transporter family of proteins, ZnT3 (Palmiter et al., 1996). ZnT3 contains six transmembrane domains circling a central pore through which Zn ions are actively pumped in exchange for hydrogen ions (Gaither and Eide, 2001). Whilst ZnT3 is the only synaptic vesicle Zn transporter it is not exclusive to neuronal synapses, some glial cells express ZnT3 RNA and ZnT3 has also been reported in the testis (Palmiter et al., 1996) and more recently a variety of other organs; however ablation of the ZnT3 gene in mice only affects Zn levels in the brain thus highlighting the essential role ZnT3 plays in brain Zn regulation (Cole et al., 1999; Smidt and Rungby, 2012).

ZnT3 can exist as a functional dimer of 80kDa (monomeric ZnT3 being 40 kDa); the formation of which may be driven by the redox status of the cellular milieu (Salazar et al., 2009). In a cellular model of oxidative stress Salazar et al found ZnT3 dimerisation to be increased. Additionally, this dimerisation was instrumental in targeting ZnT3 to vesicles and in conferring resistance to zinc-induced toxicity to the cells, as evinced by the decreased vesicle targeting and heightened sensitivity to zinc levels when cells expressed mutant ZnT3 protein lacking the amino acid tyrosine at the positions required for dityrosine bond formation and subsequent dimerisation (Salazar et al., 2009). Salazar and colleagues conclude by proposing that dimeric ZnT3 is necessary for efficient synaptic zinc regulation.

The adaptor protein complex 3 (AP3) assists in stabilising ZnT3 to the vesicle membrane (Kantheti et al., 1998). In mice lacking AP3, ZnT3 was found to be reduced at the synapse and instead localised to the nuclear membrane (Kantheti et al., 1998; Stoltenberg et al., 2004). Kantheti et al. found reduced synaptic Zn yet inexplicably, Stoltenberg and authors – using a more sensitive stain - found Zn was

not depleted at the synapse, possibly due to the remaining ZnT3 and so clearly AP3 is not essential for proper vesicular function of ZnT3.

Of particular interest is the recent observation that ablation of the ZnT3 gene in mice (ZnT3 KO) caused an age-related impairment in the Morris water maze task (Adlard et al., 2010). It was therefore suggested that ZnT3 mediated synaptic zinc homeostasis is important for maintenance of cognition and that loss of ZnT3 and the consequent dyshomeostasis of synaptic zinc adversely affects memory and cognition. ZnT3 knock-out mice also showed decreases in PSD95 and dendritic spine density only after 6 months (Adlard et al., 2010). Interestingly SPP was increased after 6 months; the authors suggest this to represent a compensatory mechanism initiated in response to the deficits at the post-synaptic terminal. Together, these results provide mechanistic insights into how changes in the level of zinc at the synapse may influence cognitive function, possibly this occurs through an effect on the scaffolding of NMDA receptors by PSD95; but as yet there is little literature on any interaction between Zn and PSD95 apart from Adlard et al.

As discussed in the preceding section, Zn can promote aggregation of Aβ, tau and α-syn; thus it would seem plausible that failure in the regulatory mechanisms of synaptic zinc (i.e. ZnT3) could be a trigger for this aggregation. However there was a reduction in synaptic Aβ oligomers in hippocampal slices from mice lacking ZnT3, incubated with Aβ oligomers, compared to wild type (Deshpande et al., 2009). The authors attributed this to vesicular Zn playing a crucial role in the synaptic targeting of Aβ molecules; a hypothesis consistent with experiments published in the same paper showing Zn chelation to reduce synaptic targeting of Aβ oligomers in hippocampal slices. Further evidence for this is provided by a recent study on post mortem tissue from individuals with AD pathology in the absence of cognitive deficits. Hippocampal tissue from these cases was found to have reductions in synaptic Aβ oligomers and total Zn levels, and no reduction in ZnT3 levels, compared to AD cases (Bjorklund et al., 2012); emphasising the role of Zn homeostasis in synaptic health and cognition.

The findings of Deshpande and colleagues are supported by a study using a double transgenic mouse generated from an APP transgenic (Tg2576) and ZnT3 knock-out, in which it was found that APP over-expressing mice without ZnT3 had a markedly diminished plaque load and concentration of insoluble Aβ than APP mice expressing ZnT3 (Lee et al., 2002). This is even more striking given that ordinarily APP transgenic mice have raised ZnT3 levels compared to wild type mice (Zhang et al., 2010); and raises the possibility that removal of synaptic Zn may protect from plaque formation. However the authors (Lee et al., 2002) did not report any investigations into the behaviour of the mice used thus preventing comparison to other work on ZnT3 knockout mice showing age related cognitive decline caused by a lack of synaptic Zn (Adlard et al., 2010).

ZnT3 has been shown to be co-localised with cortical Aβ plaques in human post mortem tissue from AD cases (Zhang et al., 2008) and in the brains of APP transgenic mice (Zhang et al., 2010). The authors conclude this to be evidence that ZnT3 is directly implicit in plaque formation rather than acting indirectly through its effect on Zn levels at the synapse or merely trapped in the aggregates as cellular debris from degenerating neurons. However, Zhang and colleagues also demonstrated an upregulation of ZnT3 in the hippocampus and cortex of the same APP transgenic mice; thus it is plausible that overproduction of ZnT3 coupled with synaptic toxicity and degeneration has lead to entrapment of ZnT3 within Aβ aggregates.

Other groups have described reductions both in ZnT3 RNA (Beyer et al., 2009b) and protein expression (Adlard et al., 2010) in AD post mortem tissue. Beyer and colleagues examined ZnT3 RNA levels in all main cortical areas; the reduction reported was evident in AD cases of Braak stage IV (i.e. cases moderately affected by AD pathology) and this result was not attributable to neuronal loss. The authors speculate that this reduction could be a protective mechanism to reduce synaptic Zn levels in response to a disease related increase in synaptic Zn. In agreement with Beyer and colleagues, Adlard et al observed a decrease in ZnT3 quantity in post mortem tissue associated with

age and with a diagnosis of AD, the decline in age was mirrored in wild type mice (Adlard et al., 2010).

1.2.4 Neurotransmitter systems

A characteristic neurochemical feature of both DLB and PDD is a decrease in cholinergic function, evidenced by reduced activity of choline acetyltransferase resulting in diminished production of acetylcholine, and eventually a loss of cholinergic neurons (Francis, 2009; Perry et al., 1994; Tiraboschi et al., 2000). In the neocortex the cholinergic deficit occurs earlier than in AD and is more pronounced; additionally there is reduced cholinergic activity in the basal ganglia and the pedunculopontine pathway (Tiraboschi et al., 2002). It is thought that the loss of function in the cholinergic system could be responsible for some of the psychiatric symptoms of DLB and PDD.

1.2.5 Mitochondrial Dysfunction

Mitochondria are organelles that generate cellular energy in the form of adenosine tri-phosphate (ATP) and in neurons are found both in the soma but with significant localisation to the synaptic bulb – indeed this was how synapses were first located during the earliest electron microscopy studies (Hollenbeck, 2005). This stationing of mitochondria is vital in providing a local and immediately available energy supply for the high demands created by the intensive endocytosis, exocytosis and vesicle recycling at the synapse; additionally mitochondria are essential for sequestering Ca^{2+} and to assist in recovery from synaptic depression following an action potential (Hollenbeck, 2005).

Consequential to this necessity of synaptic mitochondria (and coupled to the extended morphology of neurites, and the inability of neurons to meet their energy needs through glycolysis alone, when oxidative phosphorylation is saturated) is a particular synaptic vulnerability to mitochondrial dysfunction (Simpson et al., 2007). It is believed that the majority of mitochondria are manufactured in the soma and transported to and from synapses in a synaptic-activity dependent fashion (primarily in response to calcium ion and glutamate levels) (Chen and Chan, 2009; Sheng and Cai, 2012).

Specialised molecular machinery, unique to neurons, exists to facilitate movement of mitochondria via axonal transport. It is worth noting that tau is a key player. *In vitro* overexpression of tau

accentuates tau binding to microtubule associated proteins (MAP) which inhibits anterograde mitochondrial transport, resulting in imbalanced retrograde transport and accumulation of mitochondria in the soma. This can be rescued by removing tau from MAP through increasing tau phosphorylation (Sheng and Cai, 2012). A mechanism evolved by mitochondria to offset the challenge of axonal transport is mitochondrial fission and fusion; a dynamic process through which mitochondria can replicate at the synapse (via fission) and limit damage (via fusion) (Knott et al., 2008)

Mouse models have shown that both Aβ and tau disrupt mitochondrial function, although they target different components, tau complex 1 and Aβ complex IV (Ittner and Gotz, 2011). Interestingly in the absence of tau the negative impact of Aβ on mitochondrial function was removed (Sheng and Cai, 2012).

The link between α-syn and mitochondrial dysfunction is not clear; an investigation by Reeve et al using post-mortem substantia nigra neurons from individuals with DLB and PD, and controls found no significant change in mitochondrion numbers in neurons containing α-syn versus those that did not; thus, despite the established role of mitochondria in synaptic failure, the connection between this role and the synaptic pathologies remains to be elucidated (Reeve et al., 2012). Pharmacological interference in oxidative phosphorylation complex 1 (in humans and animal models) produces parkinsonism symptoms and it is known that Pink1 and Parkin are important for mitochondrial health (Chen and Chan, 2009).

Familial PD can be caused by mutations in either of these genes, and sporadic PD is characterised by mitochondrial complex 1 dysfunction caused by a significant decline in substantia nigra levels of the antioxidant glutathione, partly as a result of the high susceptibility of dopaminergic neurons to oxidative damage, an inherent property of dopamine metabolism (Jomova et al., 2010).

1.3 Brain regions of interest

1.3.1 Prefrontal cortex

The PFC is the association cortex of the frontal lobe and is one of the last cortices to fully mature in humans. It has extensive connections to the rest of the brain, in particular to the temporal, parietal and occipital cortices, hippocampus, thalamus and the limbic areas. Thus enabling processing of sensory and emotive input, vital for the PFC's control of executive function, which Teffer and Semendeferi define as 'the organization of input from diverse sensory modalities, the maintenance of attention, the monitoring of information in working memory, and the coordination of goal-directed behaviors' (Teffer and Semendeferi, 2012). Additionally the different areas of the PFC have prominent connections to each other, and indeed there is much overlap in function between the different Brodmann areas that make up the PFC.

Much of the knowledge linking specific functions to a brain region comes from studies of lesions to these areas and any arising behavioural abnormalities in the subject. Lesions of the part of the PFC containing BA9 result in a loss of executive function, characterised by the inability to plan; eg. the construction or initiation of spoken or written sentences (Fuster, 2001). However other reports and insights from lesions to the PFC have suggested behavioural and emotional changes to be the primary manifestations, foremost amongst these is the case of Phineas Gage, who retained intellect and memory after massive damage to the frontal lobe – but was drastically altered in his emotional and behavioural faculties (including disinhibition and impulsiveness) (reviewed by (Cato et al., 2004)). This historic observation has been substantiated by subsequent reports including injured soldiers and patient "K.M.", who underwent a lesion of the PFC as treatment for seizures (Cato et al., 2004). Cato and colleagues suggest that the evidence accumulated to date attributes emotional and

behavioural control to the ventro-medial PFC and cognitive and executive function to the dorso-lateral PFC (including BA9).

The diagnostic criteria for DLB according to McKeith et al state a deficit of executive function as essential for the diagnosis of DLB and so BA9 is highly likely to be implicated in the etiology of this symptom of DLB (McKeith et al., 2005).

1.3.2 Cingulate gyrus

Brodmann's area 24 corresponds to the anterior cingulate gyrus, which is part of the medial cortex and a constituent brain region of the limbic system (Zilles and Amunts, 2010). BA24 participates in several major anatomical connections to the PFC and specifically BA9, in addition to significant outputs to the ventral striatum and a feedback loop via the thalamus (Tekin and Cummings, 2002).

There is extensive debate over the function of the cingulate gyrus. Studies from patients with damage to the cingulate gyrus, or generally the medial cortex, point to attention emotional processing as a major function of the gingulate gyrus. Such patients also experience difficulty with behavioural and cognitive tasks (Fuster, 2001). This is consistent with a comprehensive review of lesions to BA24, which found the most significant presentation of these lesions to be apathy – including decreased spontaneity and initiation of movement and speech (Tekin and Cummings, 2002).

Raichle reviews a number of imaging studies which have provided further insights into anterior cingulate function, including both PET imaging in humans and monkeys that has shown BA24 to be key in attention and language generation. fMRI studies have shown increased activation of the BA24 in humans performing tasks involving sustained concentration (Raichle, 1994). Precise detail of the nature of the attention mediated by BA24 is summarised by Fuster, as shifting the focus of attention from different locations in the visual field (Fuster, 2001). This is supported by evidence of the

reciprocal connections between the cingulate, and the parietal and PFC – as all three regions are highly activated in tasks replicating spatial attention (Kastner et al., 1999).

1.3.3 Parietal cortex

Brodmann's area 40 (along with area 39) forms part of the inferior parietal lobule (IPL) of the parietal cortex – as outlined in a review of Brodmann's map by Zilles and Amunts (Zilles and Amunts, 2010). The IPL has long been known to perform important roles in integrating visual, auditory and somatosensory input – this is achieved by multiple connections to other cortical regions. In particular, the IPL has significant output connections to the visual cortical areas and the prefrontal cortex and receives input from the CA1 in the hippocampus, the superior colliculus and the cerebellum (Clower et al., 2001). The IPL is heterogeneous – a characteristic reflected in these regions innervating the IPL; hippocampal input is thought to relate to spatial perception and navigation, that from the superior colliculus to visual processing and whilst input from the cerebellum is theorised to correspond to the proprioceptive aspect of motor performance adaption (Clower et al., 2001).

Recently Kenny and colleagues reported increased connectivity to the primary visual cortex in DLB patients (compared to controls) using fMRI, and suggested that intact visual cortex is required to experience visual hallucinations and that the neural-processing errors responsible for the visual processing abnormalities and symptoms in DLB must occur upstream at a 'higher' organisational level – possibly in the parietal cortex (Kenny et al., 2012).

1.3.4 Temporal Cortex

The temporal cortex includes the visual association cortex, which comprises BA21 (and BA20 and 37) (Catani et al., 2012). Temporal lobe lesions have led to this region being implicated in memory related tasks, such as the retention and perception of objects (Milner, 2003). This is supported by observations from monkeys where lesion of the temporal or parietal cortex caused either impairment of object discrimination or discrimination of spatial relationships respectively (Müller and Knight, 2006). Additionally nominal aphasia (impaired naming) and autoscopy (visual

hallucinations of one's own body) have been linked to lesions of BA21 specifically (Catani et al., 2012).

The visual association cortex has prominent connections to the occipital cortex (via the inferior longitudinal fasciculus) and the frontal and parietal cortices, via the arcuate and uncinate fasciculi (Catani et al., 2012).

1.3.5 Brain Region Summary

In summary – the prefrontal cortex (PFC) including, the superior frontal cortex, Brodmann area 9 (BA9) is responsible for executive function (Fuster, 2001); and changes are a core symptom of DLB and PDD, whilst BA24 is one of the earliest affected cortical regions in LBD. BA40 was selected as a comparison region as this region is much less affected in LBD but exhibits considerable pathology in AD (McKeith et al., 2005). The evidence for the functions of these regions support the hypothesis that DLB and PDD are executive, visuo-spatial and attentional dementias (Collerton et al., 2003); as does the involvement of these regions in the pathology and etiology of DLB and PDD.

Furthermore, all of these regions described above are included in the standard block-taking protocol for the assessment of neuropathology in aged individuals (Alafuzoff et al., 2008; Alafuzoff et al., 2012; Alafuzoff et al., 2009; Alafuzoff et al., 2006).

1.4 Hypothesis

It is not yet clear whether pathology is an independent predictor of clinical symptoms or whether synaptic dysfunction is driving synaptic pathology or *vice versa*. Using the unique and extensive cohort and associated biochemical, clinical and pathological data, we wish to investigate relationships between pathology, synaptic biochemistry and cognitive and behavioural states in DLB, PDD and AD patients. Particular focus was given to the degree of synaptic dysfunction across brain regions and diagnostic groups, to synaptic dysfunction's relationship with pathology and clinical data across brain regions, and to the relationships between different pathologies.

The over-arching hypothesis behind these studies was that changes in synaptic structure and function in particular brain regions would underlie the clinical symptoms in the combined dementia cohort. ZnT3 was selected as a protein of interest based on evidence that zinc is important for cognition and may be involved in the generation of pathology; therefore, it was predicted that deficits in ZnT3 would relate to cognitive impairment and increased pathology. The other proteins were selected to provide a representation of the two synaptic terminals (SPP and PSD95) and a neuronal count (Btub). This was in order to determination the degree of contribution made by any loss of these elements to symptoms and what the relationship was between synapses and pathology. The anticipated outcomes being an increased knowledge of the synaptic biochemistry in LBD and its relationship to pathology and symptoms, with the potential to assist in the development of biomarkers and treatment strategies.

Additionally, it was hoped to identify differences between the three dementias, both in terms of distribution and frequency of clinical symptoms, pathology and levels of synaptic proteins, and in terms of any relationships between these variables. This could be of assistance in either delineating DLB and PDD as something more than clinical constructs; or in establishing them to have common molecular underpinnings.

Using the Brains for Dementia Research (BDR) network we have assembled an unrivalled group of over 90 autopsy confirmed LBD cases with detailed serial clinical assessments in the majority of cases and detailed semi-quantitative neuropathological scores for all regions.

In order to test these hypotheses the following steps were taken;

- A description of the clinical and pathological characteristics of the cohort.
- Analysis of the relationship between behavioural, cognitive and pathological data
- A description of the synaptic biochemistry according to brain region and clinical diagnosis
- Analysis of the relationship between synaptic biochemistry and clinical and pathological data

2 Methods and Materials

2.1 Cohort description.

2.1.1 Description of the clinical and pathological data

The data available to this project can be classified into three categories; clinical, pathological and biochemical. The clinical data can be further subdivided into that of cognitive or behavioural nature. The cognitive data comprises MMSE scores – analysed independently and used to provide the basis for an additional cognitive variable, the three categories of cognitive impairment to which cases were assigned. These were;

- 'unimpaired cognition' – for cases classified by the brain bank as being clinical controls,
- 'mildly impaired cognition without dementia' – for cases with MMSE scores of 25 to 30,
- 'mildly impaired cognition with dementia' – for cases with MMSE scores from 17 to 24,
- 'moderately impaired cognition' – for cases with MMSE scores of 10 to 16,
- 'severely impaired cognition' for cases with MMSE scores of 9 or less.

These categories were primarily based a division of the cohort into terciles according to MMSE score. This created 'cut off' MMSE values of 9 and 16. However, it was felt that the two cases with a score of 29 and the two cases with a score of 30 could not be included in the same category as cases with a score of 17. Therefore, an additional group, 'mildly impaired cognition without dementia', was created to accommodate these cases and the others above 24. These category definitions are supported by published criteria (Boller et al., 2002; Reisberg et al., 1994) and guidelines on the Alzheimer's Association website (http://www.alz.org/alzheimers_disease_steps_to_diagnosis.asp).

The behavioural data comprises semi-quantitative scores for four symptoms – agitation, depression, hallucinations and persecution, for most dementia cases these originated as NPI values, which were subsequently converted to the 0-3 scale in order to achieve compatibility with cases given a score on this scale based upon either observations made by a clinician or the inclusion of a case as a control. I

would like to express my thanks to Professors Clive Ballard and Dag Aarsland and Dr Julie Vallortigara for their significant contributions to the compilation and standardisation of this clinical data.

The pathological data is likewise semi-quantitative (on a scale of 0 – none, 1 – sparse, 2 – moderate, 3 - severe) and comprises scores for plaques/Aβ pathology, tangle/tau pathology and α-synuclein pathology. This score is based upon immunohistochemical staining, some of which was undertaken specifically for this project, whilst some was diagnostic, undertaken when the case in question was received by the respective brain bank. The semi-quantitative score was primarily introduced to standardise between scorers and between methodologies. I was involved in the coordination and compilation of this data for the cohort – as well as assisting with scoring and staining. I would like to express thanks to Dr Johannes Attems, Dr Tibor Hortobagyi and Dr David Howlett for their significant contributions to this pathological data – in particular staining and scoring of sections.

The biochemical data presented in this project was obtained from homogenised frozen grey matter using Western blotting and ELISA – and from paraffin embedded sections using immunohistochemistry and was undertaken by myself.

2.1.2 Description of post-mortem tissue

Post-mortem brain tissue from DLB, PDD and AD patients with age and gender matched healthy control tissue was the main resource for this project (see table 2.1 for details). These brains have come from a variety of sources to which the author is indebted; University Hospital Stavanger, the Brains for Dementia project based at the Institute of Psychiatry (KCL), Newcastle University and the Thomas Willis Brain Collection at Oxford University.

From each brain there is a frozen hemisphere and a hemisphere fixed in formaldehyde and extensive clinical data (see preceding section). A 500mg sample of frozen tissue was taken from the pre-frontal cortex (BA9), the cingulate gyrus (BA24) and the parietal cortex (BA40) and processed for Western blot and ELISA analysis whilst paraffin fixed, wax-embedded blocks were available for immunohistochemical analysis (see following sections).

Diagnosis	Gender (M/F) %	Age at death (mean)	PMD (mean hours)	pH (mean)	Years in storage (mean)
Control (25)	60/40	79.7 ± 7.6	39.1 ± 22.9	6.47 ± 0.28	11.8 ± 5.6
PDD (34)	53/47	79.9 ± 6.0	33.5 ± 15.6	6.44 ± 034	9.4 ± 1.2
DLB (55)	58/42	81.7 ± 6.5	41.3 ± 28.0	6.37 ± 0.41	7.8 ± 4.6
AD (16)	31/69	88.0 ± 7.8	34.9 ± 23.9	6.30 ± 0.33	2.9 ± 0.8

Table 2.1; Demographic variables according to diagnosis.

2.2 Immunohistochemistry

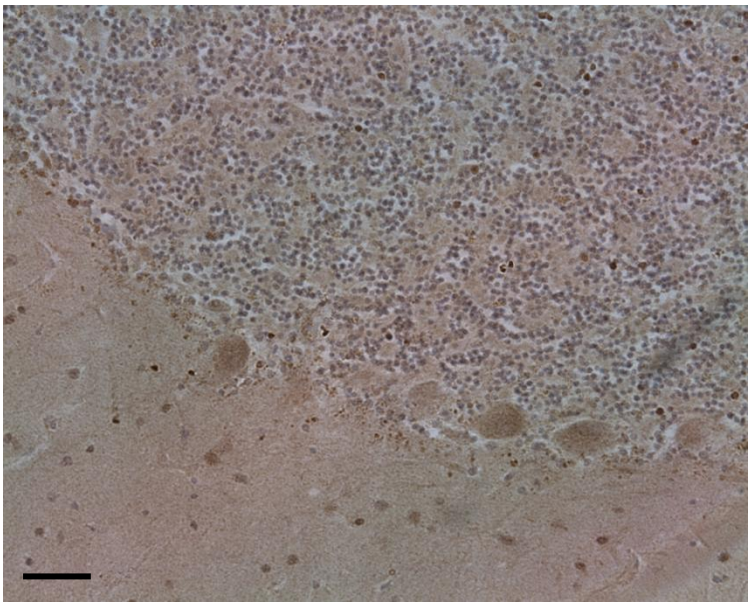
The fixed tissue was examined using immunohistochemistry (IHC) for selected proteins of interest. A standard IHC protocol was used with changes made to antibody concentration and incubation length and to the epitope retrieval technique. 7μm sections of paraffin embedded tissue were cut and de-waxed using xylene and alcohol. Epitope retrieval consisted of heating the sections in citrate buffer using a microwave to remove cross-linage of peptides caused by fixation in formaldehyde (Yamashita, 2007). The length of time and microwave power was varied to achieve the best staining (see table 2.2.1). Methanol with 0.75% hydrogen peroxide was used to quench endogenous peroxidase enzynmes present in the tissue thereby preventing a non-specific reaction with DAB (di-amino-benzidine, from Sigma). Normal serum from the same species as the secondary antibody was used to block non-specific binding of the primary antibody. A primary antibody specific to the protein of interest and a secondary antibody (from Dako) specific to IgG (immunoglobulin G) from the same species the first antibody is raised in was used to achieve specific and sensitive detection. Visualisation was achieved through a chemical reaction with DAB and hydrogen peroxide catalysed by horseradish peroxidase, which is part of an avidin-biotin complex (Vectastain ABC kit, Vector) that binds to the biotinylated secondary antibody. Sections were counter stained with haematoxylin, which stains the negatively charged DNA molecules thus showing the nuclei and hence cell bodies of the neurons. Following this sections were dehydrated with increasing concentrations of alcohol and cover-slipped using DPX mounting medium. Tris-buffered saline (TBS), pH 7.6, was used to wash the sections for 3 times for 5 minutes between each reagent.

Figures 2.2.1 and 2.2.2 show images of sections immuno-labelled for PSD95 and SPP respectively as part of the optimisation and validation of the antibodies.

Protein	Primary Antibody	Antibody Dilution	Epitope Retrieval
ZnT3	rabbit polyclonal, Synaptic Systems	1:200 overnight	extended citrate
synaptophysin	mouse monoclonal, Dako, clone SY38	1:500 overnight	normal citrate
PSD95	mouse monoclonal, Thermo Scientific, clone 6G6-1C9	1:200 overnight	extended citrate
Drebrin	mouse monoclonal, MBL, clone M2F6	1:200 overnight	extended citrate

Table 2.2.1. Proteins of interest and IHC methods.

This table shows the final antibody dilution and epitope retrieval after optimisation of staining for the proteins of interest. Extended citrate is 6 minutes at high power, 800 watts (W), then 16 minutes on low power, 240W. Normal citrate is 12 minutes at high power.



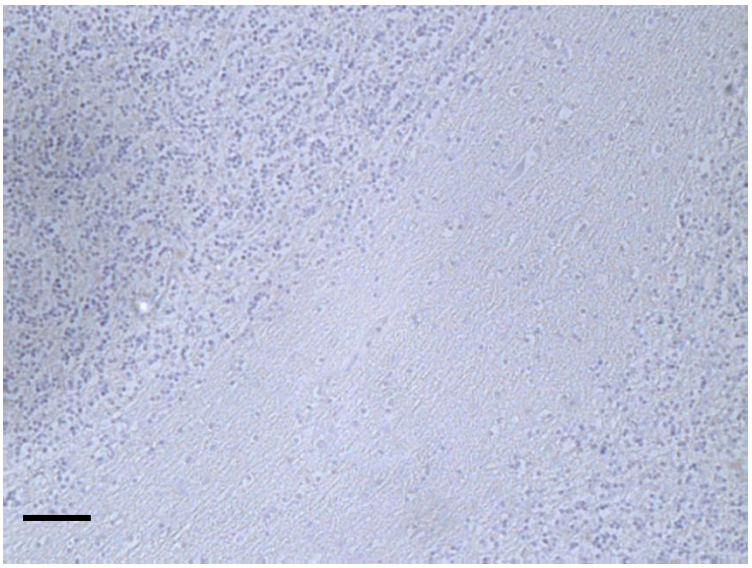


Figure 2.2.1; Cerebellar PSD95 immunohistochemistry.

The top image is a cortical section immunolabelled for PSD95. Some intensely labelled cells can be seen in the molecular layer (bottom left region of image). The Purkinje cell and granular cell layers also display positively labelled cells. The bottom image shows a negative control with no DAB labelling. Both images are cerebellum sections from an elderly healthy control case. Scale bars = 50 µm.

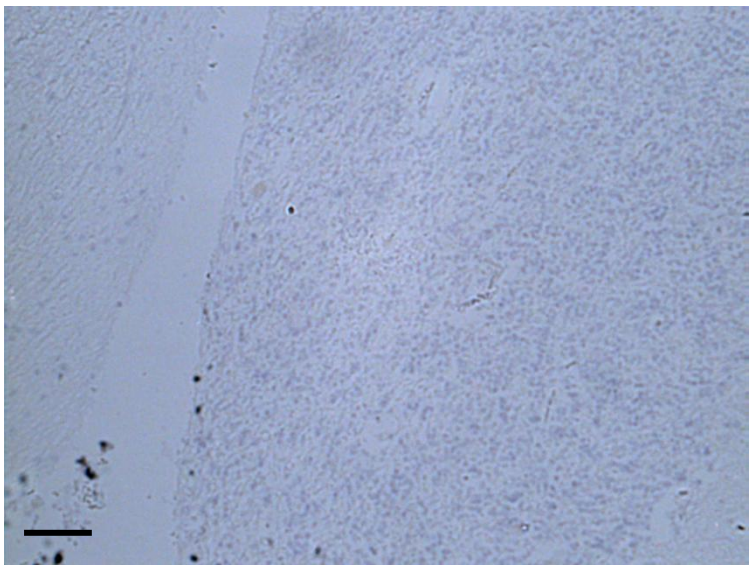


Figure 2.2.2; Cerebellar synaptophysin immunohistochemistry.

The top image shows immunopositive labelling of cells and their synapses in the molecular layer (upper right corner) and extensions in the granular layer for synaptophysin. In the bottom right of the image there is white matter without positive staining. The bottom image is a negative control showing only haematoxylin counter-stain of nuclei in all layers. Both images are cerebellum sections from an elderly healthy control case. Scale bar = 50 µm.

2.3 Tissue preparation

For each tissue sample, the cortical grey matter was dissected from the white matter and the meninges at 0°C. Approximately 300mg of cortical grey matter was homogenised in 6ml of ice-cold homogenisation buffer at pH 7.4 using an Ultra-Turrax tissue homogeniser (KIA Werke, Germany), resulting in a crude homogenate. The homogenisation buffer contained 50mM Tris-HCl, 5mM EGTA, 10mM EDTA (for chelation of magnesium and calcium ions respectively), 'Complete Protease Inhibitor Cocktail Tablets' at the appropriate dilution from Roche Applied Science (which inhibit serine, cysteine and metallo-proteases) and 2µg/mL pepstatin A (for inhibition of aspartic proteases, something not performed by the Roche tablet). This protocol is based upon that previously described in (Kirvell et al., 2006). The crude homogenates were immediately frozen on dry ice and stored at -70°C for use in protein assays and Western blotting at a later stage.

2.4 Protein Assay

The total protein concentration in the crude homogenate was determined using the Bradford protein assay method (Kruger, 1994). Before preparing the tissue samples for protein assay, standards were made by using bovine serum albumin (BSA) (Sigma-Aldrich, USA) and PBS buffer through a series of dilutions, resulting in the following concentrations; 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mg/ml. Each of these standards was then diluted by a factor of 10 whilst the samples were diluted by a factor 100 (in both cases using distilled deionised water). After the respective dilutions, 10µL of standard or sample was added to a 96-well plate (Nunc A/S, Denmark) in triplicate. Then 300µL of Coomassie protein assay reagent (Thermo Scientific, USA) was added to each well After ensuring no bubbles were present on the surface of the wells, which would interfere with the absorbance reading, the absorbance was read at 595nm using a FlexStation 3 (Molecular Devices LTD, UK). A standard curve was calculated from the standards, using GraphPad Prism version 5.0 (GraphPad Software Inc, USA), allowing the concentration of protein in each sample to be determined from the absorbance.

2.5 Semi-quantitative Western blotting

To prepare the crude homogenate for Western blotting; 250µL of 5x Laemmli sample buffer for SDS-PAGE (GenScript, USA), containing bromophenol blue and SDS, was added to 1ml of homogenate and then boiled at 95°C for 5 minutes. After cooling down, samples were stored at -20°C until Western blotting was performed.

Western blots were run in duplicate using a 10% SDS-polyacrylamide gel at approximately 160 volts (V). On each gel there were three lanes containing rat cortex, (these were always the first lane, a central lane and the last lane) and 1.5µl of full range molecular weight marker (MWM, sigma) in a central lane. Each human sample was loaded once on each of the tandem gels, the total protein concentration of each sample (obtained from the protein assay) allowed the loading volume of each sample to be adjusted such that 20µg of protein was loaded onto the gel for each sample. A similar approach was taken for rat cortex; however, when quantifying PSD95 and ZnT3, the volume of rat cortex was lowered in order to load 10µg of total protein. This was because the signal of these proteins was considerably higher in rat cortex than human cortex and therefore distorted the ratio of human cortex to rat cortex, which in turn made it harder to achieve satisfactory repetition between duplicates.

When the MWM had reached the bottom of the gel, the contents of the gel were transferred to nitrocellulose membrane (Hydrobond-C, Amersham) using semi-dry electroblotting at 60V for 90 minutes. Non-specific binding sites were blocked using a 1 hour incubation in phosphate-buffered saline (pH7.6) with 0.1% tween20 (PBST) and 5% non-fat powdered milk. Membranes were incubated overnight in the appropriate primary antibody in PBST and 5% milk (see table 2.5.1 for dilutions). Excess antibody was removed with three 5 minute washes in PBST. Membranes were incubated in PBST containing 5% milk and the relevant secondary antibody; either a donkey anti-rabbit visible under the green channel or a goat anti-mouse visible under the red channel, both IRDye from LI-COR. An Odyssey infrared scanner was used to take images of the membranes. Due to sufficient difference in molecular weight of the respective proteins, the lack of cross-reactivity

between the primary antibodies and availability of secondary antibodies fluorescent under different channels, it was possible to incubate the primary antibodies for PSD95 and ZnT3 on the same membrane; thus improving the efficiency and sample analysis times for Western blotting, illustrated in figure 2.5.1. Btub and SPP were analysed on the same membrane but antibody incubation and scanning of membranes was conducted separately, one after the other, as there was some cross-reactivity, depicted in figure 2.5.2.

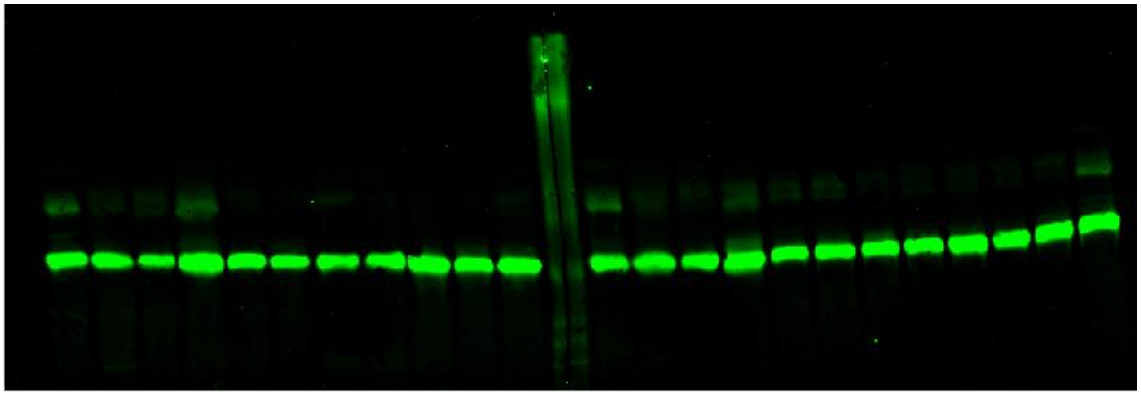


Figure 2.5.1; An example of a Western blot for Btub.

The samples run on this gel were (from left to right); rat cortex, BA9 DLB (x10), mwm, rat cortex, BA9 DLB (x2), BA24 PDD (x1), BA9 PDD (x2), BA9 DLB (x3), BA24 DLB (x2) and rat cortex.

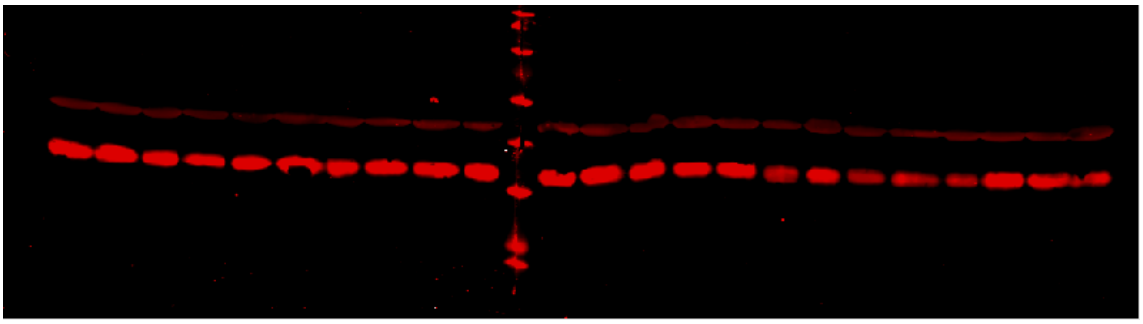


Figure 2.5.2; An example of a Western blot for SPP.

The samples run on this gel were (from left to right); rat cortex, BA24 AD (x10), mwm, rat cortex, BA24 AD (x5), rat cortex, BA9 AD (x5) and rat cortex.

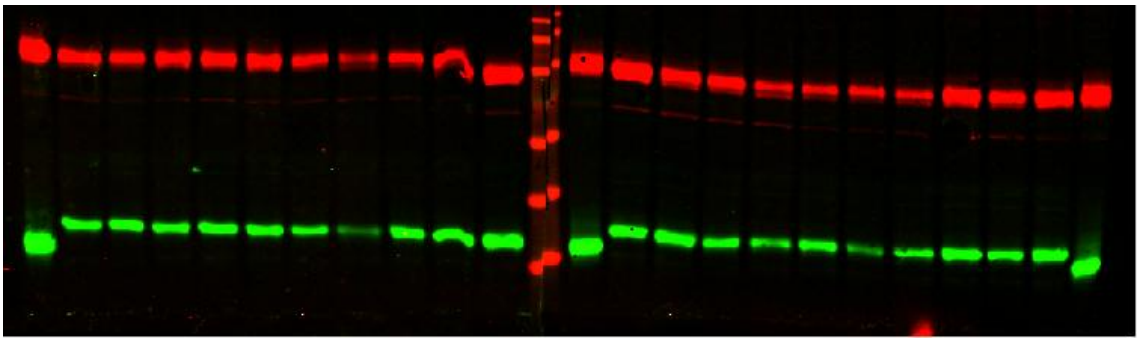


Figure 2.5.3; An example of a Western blot for PSD95 and ZnT3.

PSD95 was visible on the red channel, ZnT3 on the green channel. The samples run on this gel were (from left to right); rat cortex, BA9 AD (x3), BA24 AD (x2), BA40 AD (x5), mwm, rat cortex, BA40 AD (x1), BA40 PDD (x9) and rat cortex.

Protein	Primary Antibody	Primary Antibody Dilution
ZnT3	rabbit polyclonal, Synaptic Systems	1:1000
β-tubulin	rabbit polyclonal, Abcam	1:10,000
synaptophysin	mouse monoclonal, SY38, Abcam	1:10,000
PSD95	mouse monoclonal, Thermo Scientific, clone 6G6-1C9	1:5000

Table 2.5.1. Western blot antibodies

Antibodies and dilutions used for Western blot analysis of human samples from BA9 (prefrontal cortex) and rat cortex.

Odyssey infrared imaging systems application software version 3.0.16 was used to measure the intensity of each band on the immunoblot images. The mean intensity of the three rat cortex values was calculated, this was used to express the band intensity of the samples as a ratio to the mean rat cortex band intensity. To confirm satisfactory agreement between duplicate samples on each of the tandem membranes, samples were repeated if the percentage difference (after expression as a ratio to rat cortex) between duplicates was greater than 30%.

Due to the high number of samples analysed, more than one aliquot of rat cortex was required. To ensure continuity between aliquots of rat cortex, each antibody was quantified in the old and new rat cortex aliquot to produce a correction factor if the mean signal intensity was not the same between the rat cortices.

2.6 Enzyme-linked immuno sorbent assay (ELISA).

Preliminary western blot data indicated drebrin levels to be decreased in PDD cases, however this analysis could only be completed on 9 cases from the PDD, DLB and control groups before a new batch of anti-body was required, unfortunately this new batch did not detect human drebrin, only that in the rat cortex. Beta-III-tubulin was run at the same time to confirm the issue was not with loading or running the gel or transfer and other drebrin antibodies did not give suitable band quality for quantification, so an alternative method of quantification for drebrin was sought in order to pursue this line of enquiry. A sandwich enzyme immunoassay kit was purchased from USCN Life Sciences Inc. for drebrin (DBN1, code E92431Hu).

In line with the recommendation by the ELISA manufacturer; crude homogenate was subjected to two freeze/thaw cycles (using liquid nitrogen and a water bath at 35°C), centrifuged at 5000g and 4°C for 5 minutes, allowing the supernatant to be harvested. Two control and two PDD samples were used to optimisation sample dilution for the assay (these were selected as the preliminary Western blot data indicated controls to have the highest drebrin levels and PDD the lowest, thus it was hoped to cover a representative range of drebrin concentration). Following this it was determined that samples should be diluted 1:25.

A blank, 7 standards (10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.312ng/ml and 0.156ng/ml) and samples were run in duplicate. Standards and samples were incubated in the pre-coated wells for 2 hours (all incubations were at 37°C), then incubated with the first detection reagent for 1 hour. Next the wells were washed thoroughly, the second detection reagent was incubated in the wells for 30 minutes, washed out, then substrate and finally stop solutions were added. After completion of the assay, the 96-well plate was read at 450nm on a FlexStation 3 (Molecular Devices LTD, UK) and the mean concentration of each sample was calculated from the standard curve.

In order to account for varying protein concentrations between samples, a protein assay was performed on the supernatant for each sample (see protein assay section under Western blotting for details). This allowed the drebrin concentration calculated from the ELISA to be expressed as ng of drebrin per μg of total protein.

2.7 Paraffin-Embedded Tissue Blotting (PET blot)

This is a technique designed to reveal the localisation of protein aggregates within paraffin embedded tissue (Kramer and Schulz-Schaeffer, 2007). Unfortunately, it was not possible to develop the technique beyond an experimental stage. Presented here is a description of the optimisation attempts and a brief discussion of some problem solving measures undertaken.

Sections were cut on a microtome (3μm) from the amygdala of a Lewy body disease case and from the cerebellum of a healthy control brain and placed in a water bath. Alcohol (20%) was added to the water bath to improve the surface tension, making it easier for the very thin sections to be mounted on strips of nitrocellulose membrane (Hyrdobond-C, Amersham). The membranes were left to dry at 60°C for at least 24 hours.

The sections were de-waxed using xylene and hydrated with decreasing concentrations of isopropanol (from 100% down to 25%). Isopropanol was used because ethanol causes permanent curling of the membranes. The sections were incubated overnight at 60°C in TBS containing 0.1% tween20 (TBST) and different concentrations (5-250μg/ml) of proteinase K (Bioline). Sections were denatured in 4M guanidinethiocynate (15 minutes), blocked for non-specific binding in TBST with 0.2% casein, and then incubated in primary antibody (Invitrogen, mouse monoclonal anti-α-syn, clone LB509) at different dilutions (from 1:5000 to 1:1000) for 90 minutes.

The secondary antibody (incubation 1:1000 for 60 minutes) was an alkaline-phosphatase conjugated goat anti-mouse IgG. Prior to application of the visualisation reagents, sections were washed with NTM solution (100nM Tris HCl, 100mM NaCl, 50mM $MgCl_2$, pH 9.5) to raised the pH. The detection system used was the formazan reaction, NBT BCIP (nitro-blue tetrazolium chloride and

5-bromo-4-chloro-3`-indoyl-phosphate-p-toluidine salt) in a combined liquid substrate system from Sigma. Finally, sections were washed in 0.025M EDTA to strengthen the signal before being left at room temperature (in a light proof box) to dry. As the sections are opaque standard light-microscopy could not be used and, a high-magnification dissecting microscope being unavailable, a powerful dissecting torch had to be shone onto the sections when they were under a standard microscope lens.

The two major issues encountered whilst optimising the PET blot technique were the proteinase K digestion and visualising the sections under a microscope. The proteinase K concentration of 250µg/ml used by Kramer and Schulz-Schaeffer is unusually high, in comparison to other applications. This necessitated making the solution from powdered enzyme; however, the high static charge of proteinase K made accurate weighing (despite use of a static-gun) difficult. To combat this a wide range of concentrations were tried to eliminate the possibility of an erroneously high solution causing excessive digestion of the tissue section. The other problem with regards to proteinase K was the use of the so called 'pillow technique', developed by Kramer and Schulz-Schaeffer. This was not adequately described, in the original article or email communication with the authors, to be replicated. Nor was its purpose fully explained, as immersion of the sections in the digestion buffer should have achieved the same result as sandwiching the sections in paper towel soaked in digestion buffer.

Visualisation of the sections, upon completion of the protocol, was difficult due to their opacity and the impossibility of retaining a totally flat membrane surface; this meant the microscope could not focus on the entire field of view as there were in effect, troughs and peaks in the membrane surface (see figures 2.7.1 and 2.7.2). Whilst optimisation was ongoing, a critique of the technique published by Kramer and Schulz-Schaeffer was published suggesting improvements, including use of methanol to make the membranes transparent and suitable for conventional light-microscopy (Moh et al., 2010).

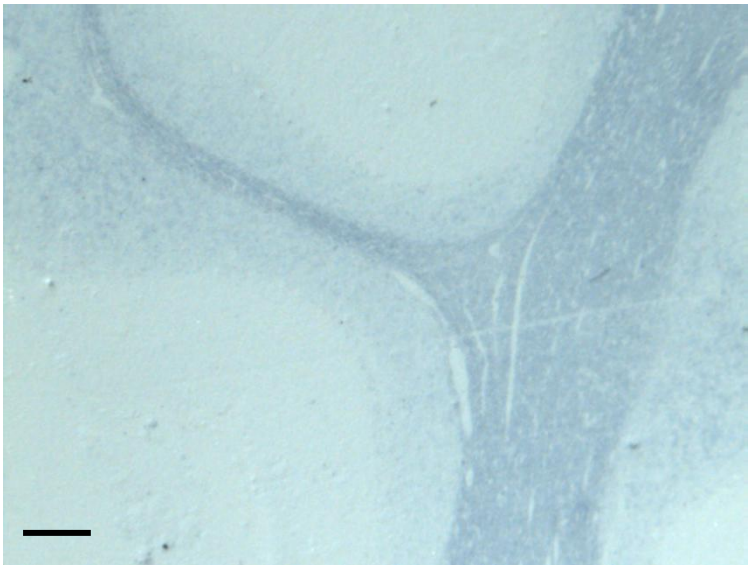
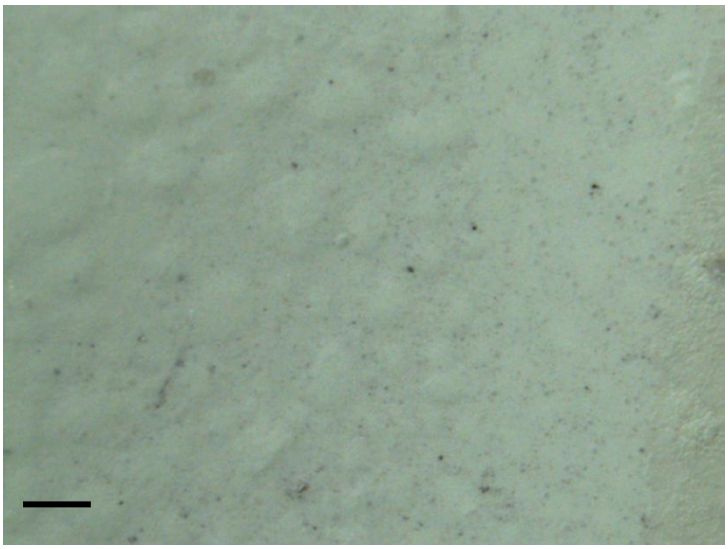


Figure 2.7.1; PET blotting in the cerebellum.

The cerebellum serves as a 'negative control' (because no ASyn pathology has been reported in the cerebellum in PDD and DLB). Both images are from a healthy control, digested with 60µg/ml proteinase K, immuno-labelled with 1:1000 anti-ASyn antibody and stained using the formazan reaction. The top image was counter stained with haematoxylin (to better reveal the tissue structure) and the bottom image was not. Scale bars = 100 µm.



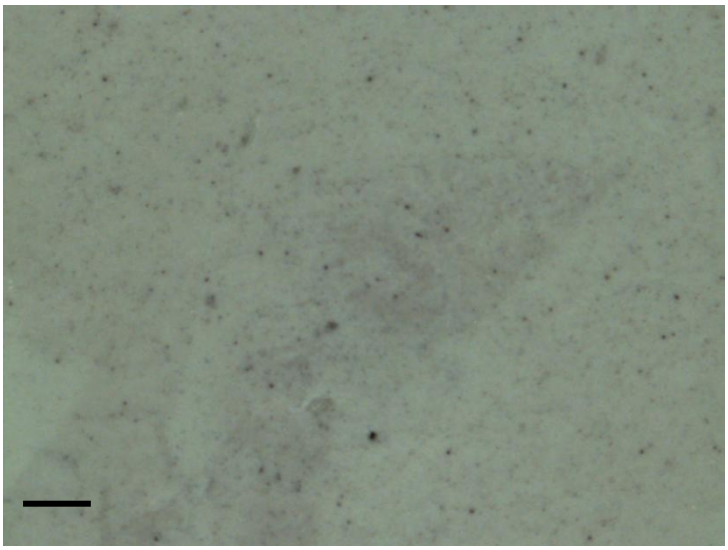


Figure 2.7.2; PET blotting in the amygdala.

Both images are amygdala sections from a case diagnosed with Lewy body dementia. The top image is a section not treated with proteinase K, the bottom image is a section that was treated with 30µg/ml proteinase K. Both were immuno-labelled with 1:500 anti-ASyn antibody and stained using the formazan reaction. Scale bars = 50 µm.

3 Statistical analysis

3.1 Demographic variables

There are a variety of factors – beyond the control of investigators - that can influence protein expression in frozen brain tissue, and which can be broadly split into those of a demographic nature (age at death and gender) and those connected to the tissue donation process (post-mortem delay (PMD), brain tissue pH and years in storage). Gender-specific differences within the brain can arise as a result of the interactions of sex-steroids such as estrogen (Kelly et al., 1999) – recently it has been demonstrated that such interactions exert an influence on the morphology and function of dendritic spines (Rasia-Filho et al., 2012). The potential impact of age on synaptic proteins requires less evincing - age being the most prominent risk factor for dementia and commonly associated with cognitive decline (Ballard et al., 2011b; DeCarli et al., 2012).

The rationale behind the creation of 'years in storage' as a variable is described under synaptophysin in the discussion. The pH in brain tissue is known to be unchanged across all brain regions and is not significantly affected by frozen storage duration (Johnston et al., 1997); furthermore, pH is a crucial confounding factor to be considered as it provides a measure of agonal state – in particular low pH is associated with prolonged death and reductions in RNA and protein quality (Johnston et al., 1997).

The effect of post-mortem delay on synaptic proteins in frozen human brain tissue was investigated by Siew and colleagues, and was found to vary according to protein and brain region (Siew et al., 2004). This is discussed further under synaptophysin and PSD95 in the discussion, but serves here as evidence of the importance of considering – and statistically controlling for – the effect of PMD. Thus, the necessity of determining the presence of any relationship between the proteins measured in this project and these confounding variables is established. Table 2.1 shows the average values for these confounding factors, according to diagnosis.

There are other potential confounding variables specific to post-mortem studies on neurodegenerative conditions. In the case of this study these are the duration of dementia, duration

of Parkinsonism and medication. In the case of the first two, it was felt that the nature of the diagnosis of DLB and PDD is such that clinical diagnosis has already taken into account the duration of dementia or Parkinsonism. This was confirmed when creating residual synaptic protein values accounting for these variables merely removed the differences in protein levels between DLB, PDD and controls.

However, certain medication can influence synaptic and neuronal proteins (further detail is given in the discussion sections for the relevant synaptic proteins). Unfortunately, data was not available on the medication taken by the AD cases and certain DLB and PDD cases, making it impossible to reliably elucidate any effect on the proteins of interest by medication. The medication appendix table shows what data was available, from which it can be seen that medication was broadly similar across DLB and across PDD patients.

3.1.1 Statistical preparation of semi-quantitative Western blotting values for β-III-tubulin, PSD95, synaptophysin and ZnT3, and the ratio of synaptophysin to β-III-tubulin, PSD95 to β-III-tubulin and ZnT3 to synaptophysin in BA9.

The normality of the data for each protein or ratio was determined using the Shapiro-Wilk test in SPSS, this test is the most appropriate for data sizes up to n=2000 (Coolican, 2009). Then the relationships between protein values or ratios and confounding variables was determined using Spearman's rank correlation in SPSS to search for significant correlations between the proteins measured by semi-quantitative Western blot in each brain region – and gender, age at death, PMD, pH and years in storage. Table 3.1.1 shows the significant correlations that occurred in BA9 between; PSD95 values and PMD, SPP values and years in storage, ZnT3 values and gender, the ratio of PSD95 to Btub and PMD and the ratio of ZnT3 to SPP and gender and pH. For full details of all values refer to appendix tables 'Protein values from semi-quantification of Western blotting', 'Residual and normalised protein values in BA9' and 'Residual and normalised protein ratios in BA9'. For all statistical analysis in this project $p<0.05$ was considered as being statistically significant.

BA9	Age at death	Gender	Brain tissue pH	Post-mortem delay	Years in storage
Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
PSD95	None (p>0.05)	None (p>0.05)	None (p>0.05)	**p<0.05**	None (p>0.05)
SPP	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	**p<0.05**
ZnT3	None (p>0.05)	**p<0.05**	None (p>0.05)	None (p>0.05)	None (p>0.05)
Ratio of SPP to Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	**p<0.05**
Ratio of PSD95 to Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	**p<0.05**	None (p>0.05)
Ratio of ZnT3 to SPP	None (p>0.05)	**p<0.05**	**p<0.05**	None (p>0.05)	None (p>0.05)

Table 3.1.1; Correlations between biochemical and demographic data in BA9

Spearman's rank correlation was used to determine the effect of demographic factors (age at death and gender) and variables associated with the tissue donation process (pH, PMD and years in storage) on the protein values and ratios obtained or calculated from semi-quantitative Western blotting. The significance levels of the relationship between these protein values (Btub, PSD95, SPP and ZnT3) and ratios (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) in BA9 and the aforementioned variables are shown in the table.

The confounding variables found to correlate with protein values were entered into a regression analysis (using the enter method in SPSS) to determine if they were significant predictors of the protein value to which the correlation occurred. If this proved to be the case then unstandardised residuals were saved from the regression, if not the case then any non-significant predictors were removed and the regression redone. If necessary, the residual variables were normalised using a transformation. For the sake of consistency, the different transformation operations were attempted in the same order; log10, square root, and quantile. Whenever possible the same operation was used for all data sets in a brain region – as these were analysed together, however quantile transformation is an exception to this – this is discussed under statistical preparation for BA24. As residual variables typically contain negative values it was necessary to shift all values above 0, usually by adding 1 to all values, before operations such as log10 could be performed.

In BA9 the final values used for each protein and protein ratio in all subsequent analysis were as follows;

- β-III-tubulin – not a residual but normalised using log10
- PSD95 – residual for prediction by PMD
- synaptophysin – residual for prediction by 'years in storage' and normalised using log10
- ZnT3 – residual for prediction by gender
- Ratio of synaptophysin to β-III-tubulin – residual for prediction by 'years in storage'
- Ratio of PSD95 to β-III-tubulin – residual for prediction by PMD
- Ratio of ZnT3 to synaptophysin – residual for prediction by gender

3.1.2 Statistical preparation of semi-quantitative Western blotting values for β-III-tubulin, PSD95, synaptophysin and ZnT3 in BA24.

The normality of the data for each protein and ratio obtained or calculated from semi-quantitative Western blotting in BA24 was determined using the Shapiro-Wilk test in SPSS. It was found that none of the protein values or ratios were normally distributed. Transformation using Log10 normalised the distribution of Btub and ZnT3 data but not SPP or PSD95 data. A square root transformation normalised PSD95, SPP was left without a normal distribution at this stage as conventional means did not achieve normalisation. The ratio of SPP to Btub and ZnT3 to SPP were normalised by taking the log10, the ratio of PSD95 to Btub was normalised by taking the square root. Following these normalisations, a number of correlations were found between the protein data or ratios and the key demographic variables; gender, age at death, brain tissue pH and post mortem delay, shown in table 3.1.2. For full details of all protein values refer to appendix tables 'Protein values from semi-quantification of Western blotting', 'Residual and normalised protein values in BA24' and 'Residual and normalised protein ratios in BA24'.

BA24	Age at death	Gender	Brain tissue pH	Post-mortem delay	Years in storage
Btub	None (p>0.05)	**p<0.05**	None (p>0.05)	None (p>0.05)	None (p>0.05)
PSD95	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
SPP	None (p>0.05)	None (p>0.05)	None (p>0.05)	**p<0.05**	None (p>0.05)
ZnT3	**p<0.05**	**p<0.05**	**p<0.05**	None (p>0.05)	None (p>0.05)
Ratio of SPP to Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
Ratio of PSD95 to Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
Ratio of ZnT3 to SPP	**p<0.05**	None (p>0.05)	**p<0.05**	**p<0.05**	None (p>0.05)

Table 3.1.2; Correlations between biochemical and demographic data in BA24.

Spearman's rank correlation was used to determine the effect of demographic factors (age at death and gender) and variables associated with the tissue donation process (pH, PMD and years in storage) on the protein values and ratios obtained or calculated from semi-quantitative Western blotting. The significance levels of the relationship between these protein values (Btub, PSD95, SPP

and ZnT3) and ratios (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) in BA24 and the aforementioned variables are shown in the table.

Residual variables were created using the approach outlined for BA9. In BA24 the final values used for each protein and protein ratio in all subsequent analysis were as follows;

- β-III-tubulin – residual for prediction by gender
- PSD95 – not a residual but normalised using the square root
- synaptophysin – residual for prediction by PMD and normalised using quantiles
- ZnT3 – residual for prediction by age at death and pH
- Ratio of synaptophysin to β-III-tubulin – not a residual but normalised using log10
- Ratio of PSD95 to β-III-tubulin – not a residual but normalised using the square root
- Ratio of ZnT3 to synaptophysin – a residual for prediction by PMD and pH and normalised using quantiles

A quantile transformation was necessary to normalise the SPP residual variable and the ratio of ZnT3 to SPP residual, in part due to the bimodal nature of these data. As this is a more advanced operation beyond the capabilities of SPSS, it was undertaken by Dr Stephen Newhouse, the statistician assisting with this project, using R software facility from Free Software Foundation, Inc. 59 Temple Place, Suite 330, Boston, USA.

3.1.3 Statistical preparation of semi-quantitative Western blotting values for β-III-tubulin, PSD95, synaptophysin and ZnT3 in BA40.

Normality tests were undertaken as described in the previous sections, followed by the establishment of any correlations to demographic and other confounding factors – the result of this is displayed in table 3.1.3. For full details of all protein values refer to appendix tables 'Protein values from semi-quantification of Western blotting', 'Residual and normalised protein values in BA40' and 'Residual and normalised protein ratios in BA40'.

BA40	Age at death	Gender	Brain tissue pH	Post-mortem delay	Years in storage
Btub	**p<0.05**	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
PSD95	**p<0.05**	None (p>0.05)	None (p>0.05)	**p<0.05**	None (p>0.05)
SPP	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
ZnT3	**p<0.05**	**p<0.05**	**p<0.05**	**p<0.05**	None (p>0.05)
SPP to Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)	None (p>0.05)
PSD95 to Btub	None (p>0.05)	None (p>0.05)	None (p>0.05)	**p<0.05**	None (p>0.05)
ZnT3 to SPP	None (p>0.05)	**p<0.05**	**p<0.05**	None (p>0.05)	None (p>0.05)

Table 3.1.3; Correlations between biochemical and demographic data in BA40.

Spearman's rank correlation was used to determine the effect of demographic factors (age at death and gender) and variables associated with the tissue donation process (pH, PMD and years in storage) on the protein values and ratios obtained or calculated from semi-quantitative Western blotting. The significance levels of the relationship between these protein values (Btub, PSD95, SPP and ZnT3) and ratios (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) in BA40 and the aforementioned variables are shown in the table.

Residual variables were created using the approach outlined for BA9. In BA40 the final values used for each protein and protein ratio in all subsequent analysis were as follows;

- β-III-tubulin – residual for prediction by age at death
- PSD95 – residual for prediction by PMD
- synaptophysin – no transformation or residual
- ZnT3 – residual for prediction by PMD
- Ratio of synaptophysin to β-III-tubulin – not a residual but normalised using log10
- Ratio of PSD95 to β-III-tubulin – a residual for prediction by PMD
- Ratio of ZnT3 to synaptophysin – a residual for prediction by gender

3.1.4 Statistical analysis outline

Advice on statistical procedure was gratefully received from a statistician – Dr Stephen Newhouse – who has been associated with this project. This consisted of guidelines on the creation of residuals and the use of multiple regression in SPSS. The approach used for analysis of relationships between the variables of interest was the same as that applied to the creation of residuals. Spearman's correlation and/or one-way ANOVAs were used to screen for significant differences. Variables that had significant differences between groups were entered into regression analysis.

4 Results

When the antibody signal was quantified on the membrane, the values for each protein were expressed as ratios to rat cortex, however, the values used in all analysis and figures have been subjected to further analysis including normalisation and the creation of residuals. To avoid repetitive complexities in the figure captions these have been comprehensively detailed in the methods section, thus all subsequent reference to the values of a protein or protein ratio refers to the final output values from these statistical computations and not the initial quantification from Western blotting.

Furthermore, the number of cases analysed and depicted in the graphs varies for each protein and brain region. Scatter plots have been used to allow a visual approximation of the n for each figure to be arrived at by the viewer, for the complete description the relevant appendix tables should be referred to.

Some of these final residual variables contained negative values, to allow easy visual comparison of the data when depicted as scatter plots, the decision was taken to translate any variables that contained negative values by an amount sufficient to ensure the largest negative value became positive. An advantage of this is that the data points do not risk being obscured by their original spread across the x axis, and the mean bars do not fall on the x axis.

4.1 Clinical and pathological data

As described under the methods and materials section, the cases used in this study included semi-quantitative scores (on a scale of 0 - absent, 1 – sparse or mild, 2 - moderate and 3 - frequent or severe) for Aβ, tau and α-synuclein pathology in BA9, BA24, BA21 and BA40. Figures 4.1.1 to 4.1.4 illustrate the distribution of these three pathologies, by severity and according to brain region and diagnosis. Figures 4.1.5, 4.1.6 and 4.1.7 show examples of the four categories of staining severity for Aβ, tau and α-syn respectively. It can be seen in figure 4.1.1 that control cases had a complete absence of α-syn pathology in all brain regions; tau pathology was likewise relatively sparse, whilst Aβ pathology was more common, with a few cases in the severe category. PDD cases were similarly characterised by a relative scarcity of α-syn, except in BA24, and, across all regions, tau (depicted in figure 4.1.2). In DLB cases there was a considerably greater frequency of cases with moderate and severe scores for all pathologies (figure 4.1.3); moreover the majority of AD cases had frequent/severe scores for Aβ and tau pathology (except in BA24 where cases were spread evenly across the 4 score categories) but a paucity of α-syn pathology (figure 4.1.4).

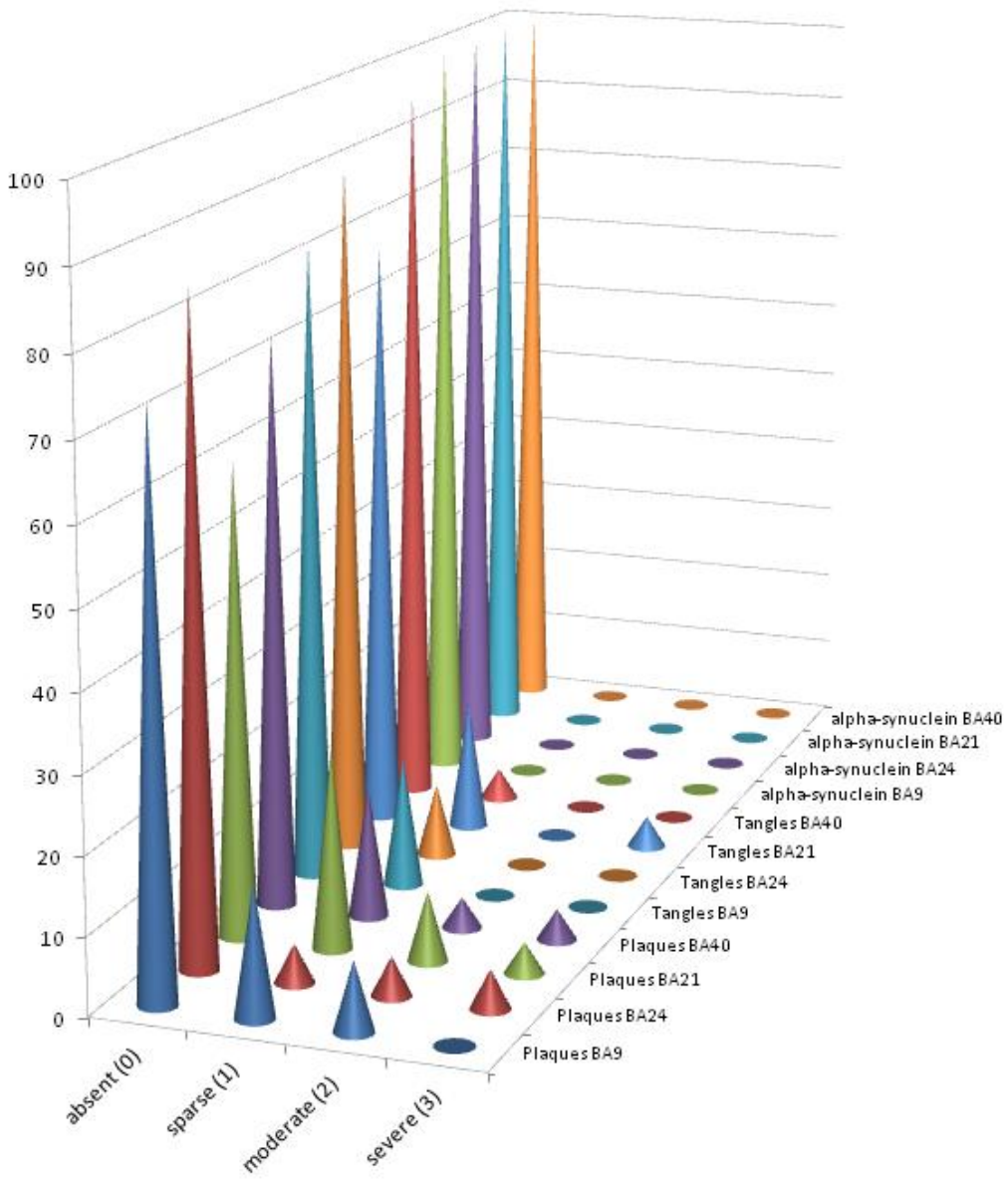


Figure 4.1.1 Frequency of pathology scores in Control cases

The frequency of each pathology score, by brain region, was calculated for those control cases that had scores and represented graphically. The left-hand y axis represents the percentage of cases with a score.

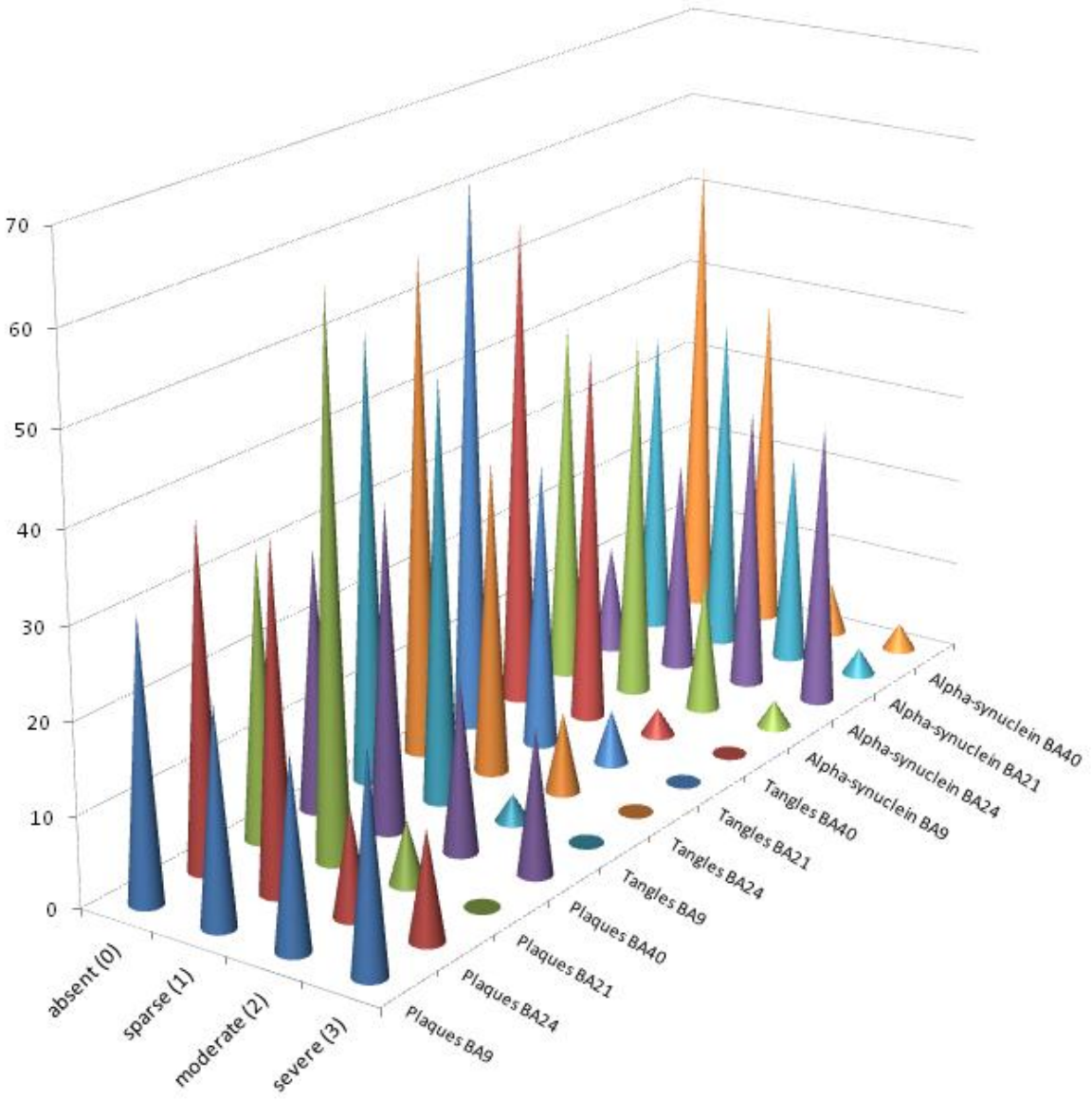


Figure 4.1.2 Frequency of pathology scores in PDD cases

The frequency of each pathology score, by brain region, was calculated for those PDD cases that had scores and represented graphically. The left-hand y axis represents the percentage of cases with a score.

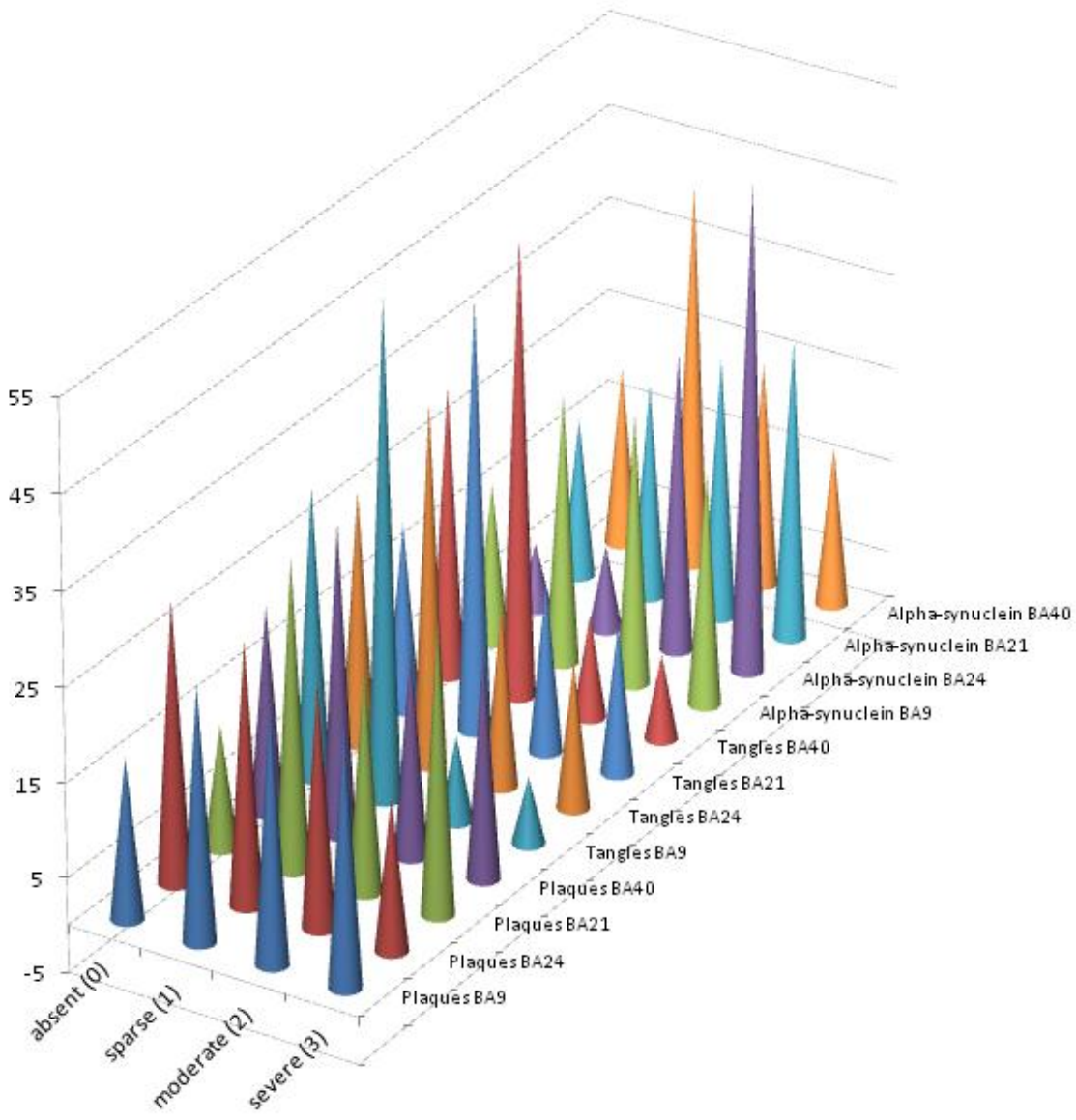


Figure 4.1.3 Frequency of pathology scores in DLB cases

The frequency of each pathology score, by brain region, was calculated for those DLB cases that had scores and represented graphically. The left-hand y axis represents the percentage of cases with a score.

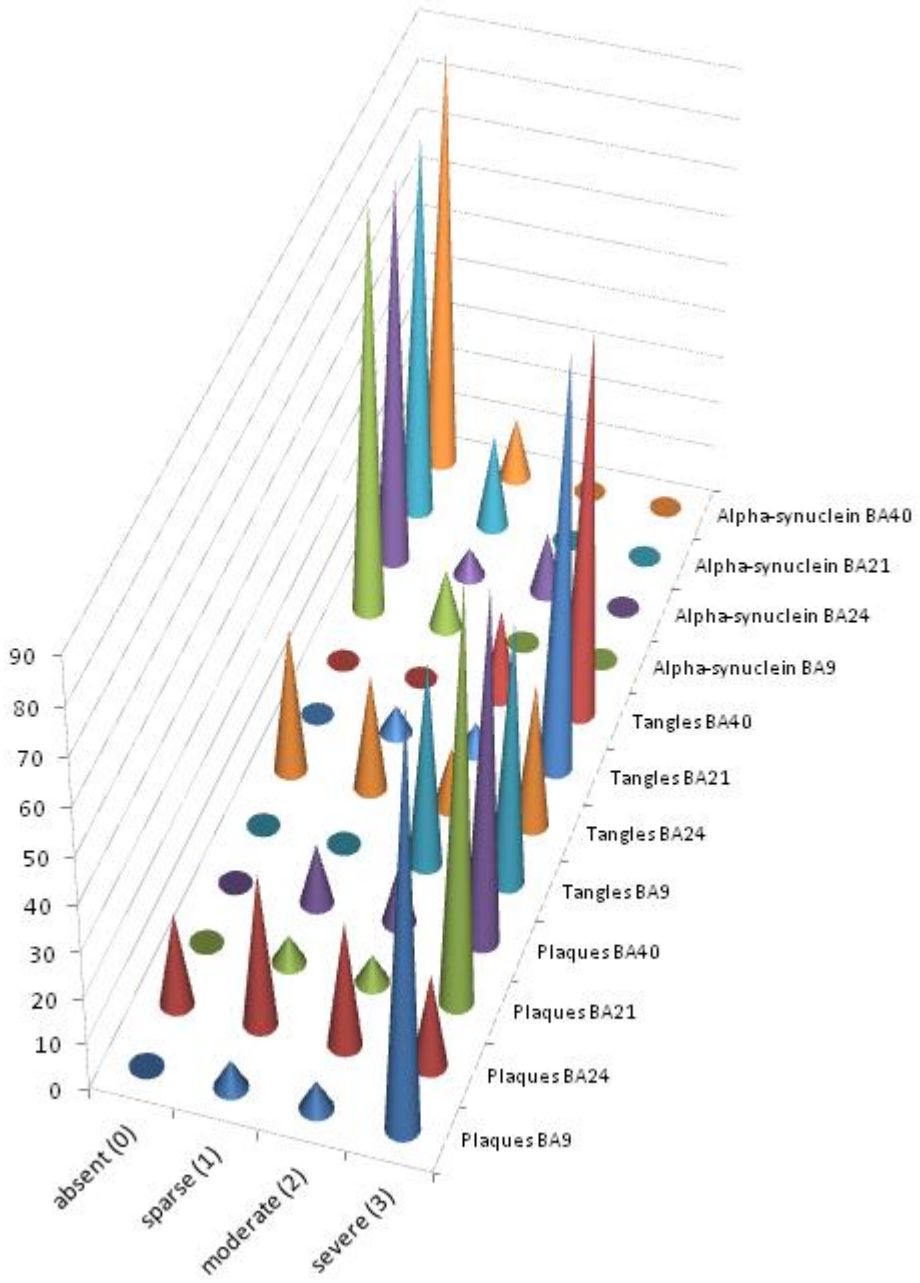


Figure 4.1.4 Frequency of pathology scores in AD cases

The frequency of each pathology score, by brain region, was calculated for those AD cases that had scores and represented graphically. The left-hand y axis represents the percentage of cases with a score.

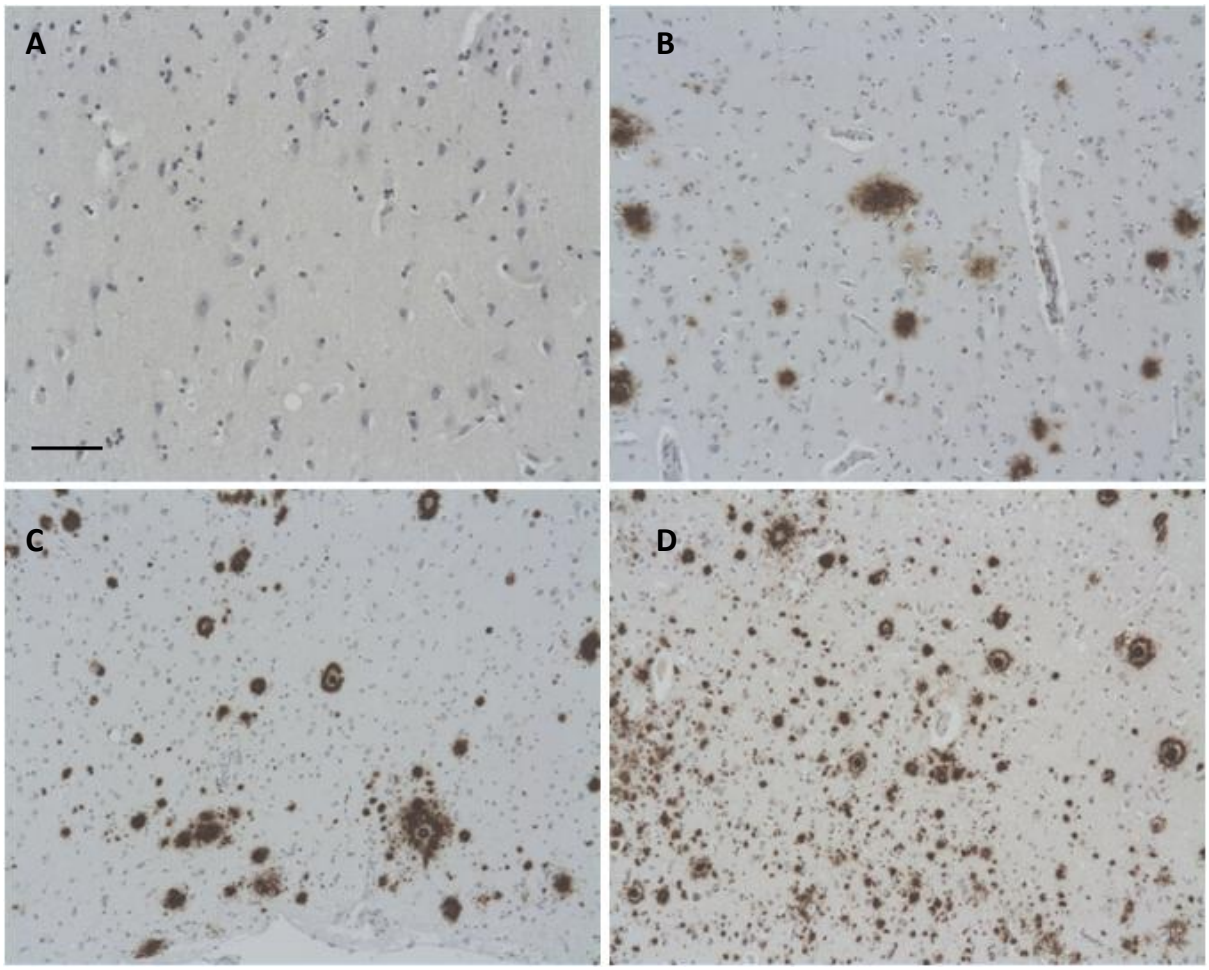


Figure 4.1.5; Images of Aβ staining representative of the four categories of semi-quantitative score.

Immuno-labelling with a 1E8 antibody for Aβ at 1:1000 was used to reveal Aβ pathology. Whilst the whole section was viewed to determine the semi-quantitative score, representations of each score category are shown here; image A is from a case given a score of absent/0, image B is from a case given a score of sparse/1, image C is from a case given a score of moderate/2 and image D is from a case given a score of severe/4. The horizontal bar is 50µm.

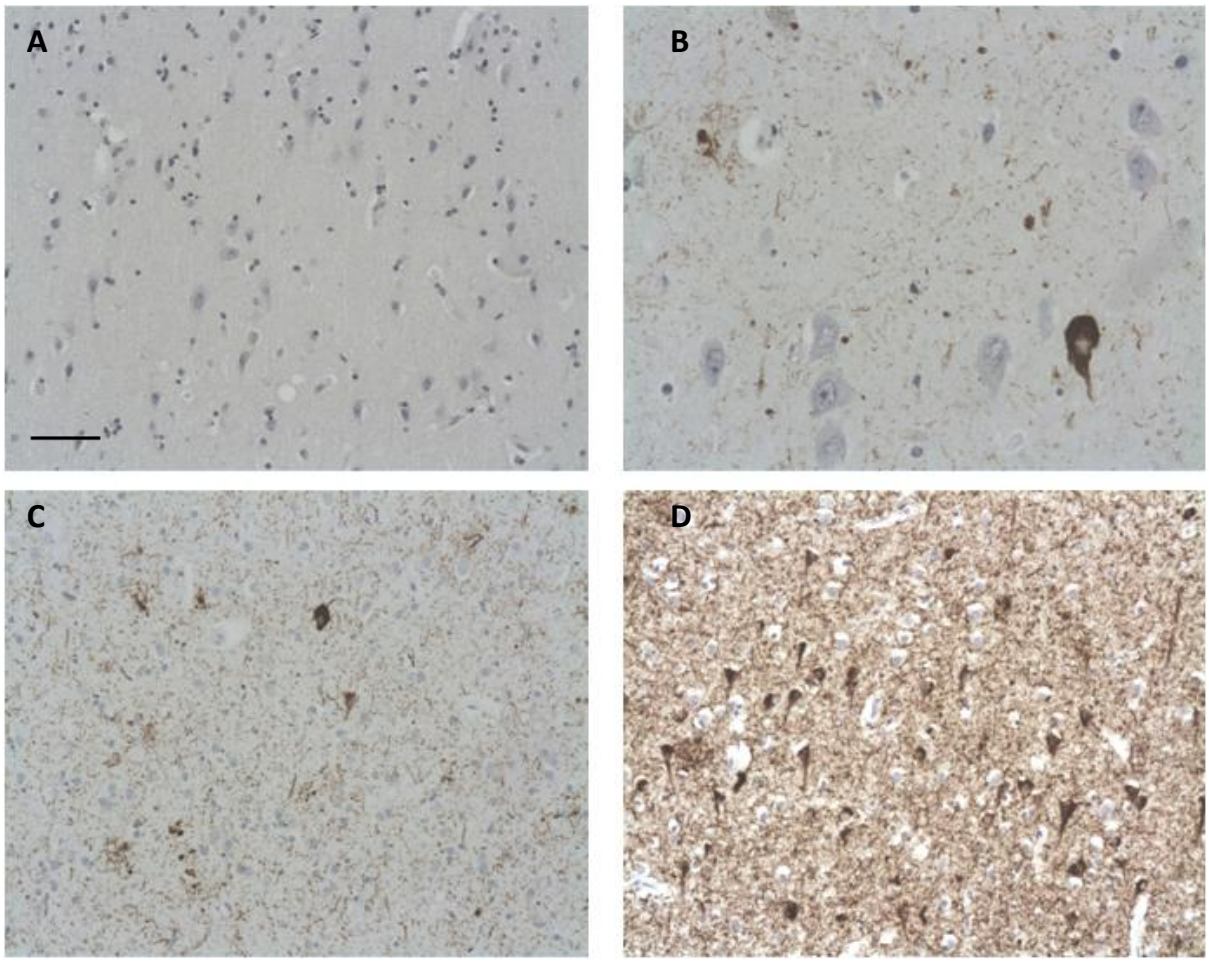


Figure 4.1.6; Images of tau staining representative of the four categories of semi-quantitative score.

Immuno-labelling with a AT8 antibody (Innogenetics) at 1:200 was used to reveal tau pathology. Whilst the whole section was viewed to determine the semi-quantitative score, representations of each score category are shown here; image A is from a case given a score of absent/0, image B is from a case given a score of sparse/1, image C is from a case given a score of moderate/2 and image D is from a case given a score of severe/4. The horizontal bar is 50µm.

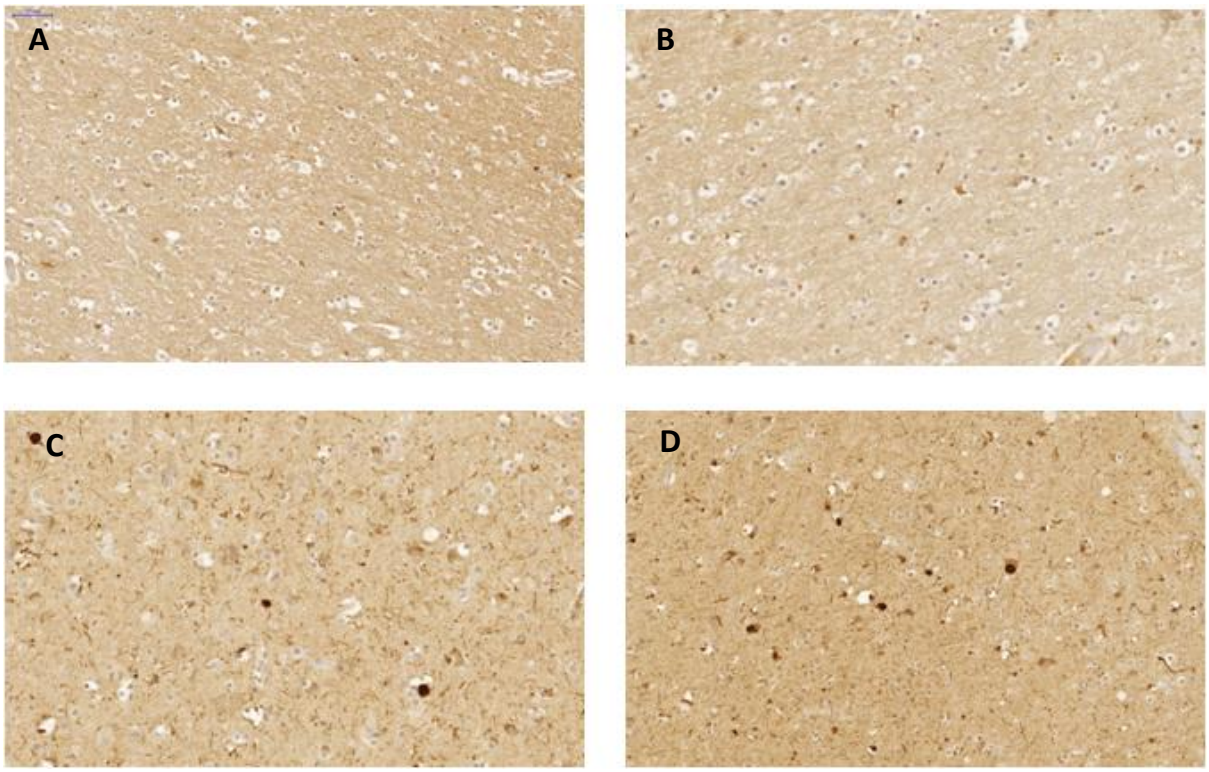


Figure 4.1.7; Images of α-synuclein staining representative of the four categories of semi-quantitative score.

Immuno-labelling with a NCL-SYN antibody (Novacastra Laboratories) at 1:200 was used to reveal α-synuclein pathology. Whilst the whole section was viewed to determine the semi-quantitative score, representations of each score category are shown here; image A is from a case given a score of absent/0, image B is from a case given a score of sparse/1, image C is from a case given a score of moderate/2 and image D is from a case given a score of severe/4. The horizontal bar is 50µm.

The clinical data available to this project can be divided into that of a cognitive or behavioural nature. The behavioural data comprises semi-quantitative scores (on a similar scale to that of the pathology) for agitation/aggression, depression, hallucinations and persecution. These have been derived from a combination of NPI scores, case notes from clinicians or inclusion as a clinical control by the brain bank. Thus, control cases were assumed to have an absence of all behavioural symptoms (illustrated in figure 4.1.5). The same figure shows the frequency of the four symptoms across the other diagnostic groups, where it can be seen that AD cases had relatively high agitation scores, which was less so for DLB cases, with PDD cases falling between the two. Depression scores were particularly low in DLB cases, with a fairly even spread for AD and PDD. However, AD cases had considerably lower hallucination scores than DLB or PDD cases. Cases of all three dementias were fairly evenly distributed across the categories of persecution scores. Nonetheless, it should be noted a considerable percentage of cases of each dementia had a score of absent for each symptom.

The cognitive data was in the form of MMSE scores, which were not available for control cases. In order to included controls in the analysis of cognition, five categories of cognitive impairment were created, based upon either the final MMSE score before death, or the inclusion in the brain bank of a case as a clinical control (see the relevant methods and materials section for further details). Figure 4.1.6 shows the distribution of cases, according to clinical diagnosis, amongst these cognitive impairment categories. The highest frequency of cases classified as severely impaired were AD, furthermore there were no AD cases classified as belonging to the MCI category. Interestingly, more DLB cases had moderate impairment than severe impairment. The percentage of PDD cases in increased as impairment increased. Finally, control cases formed the entirety of the control category.

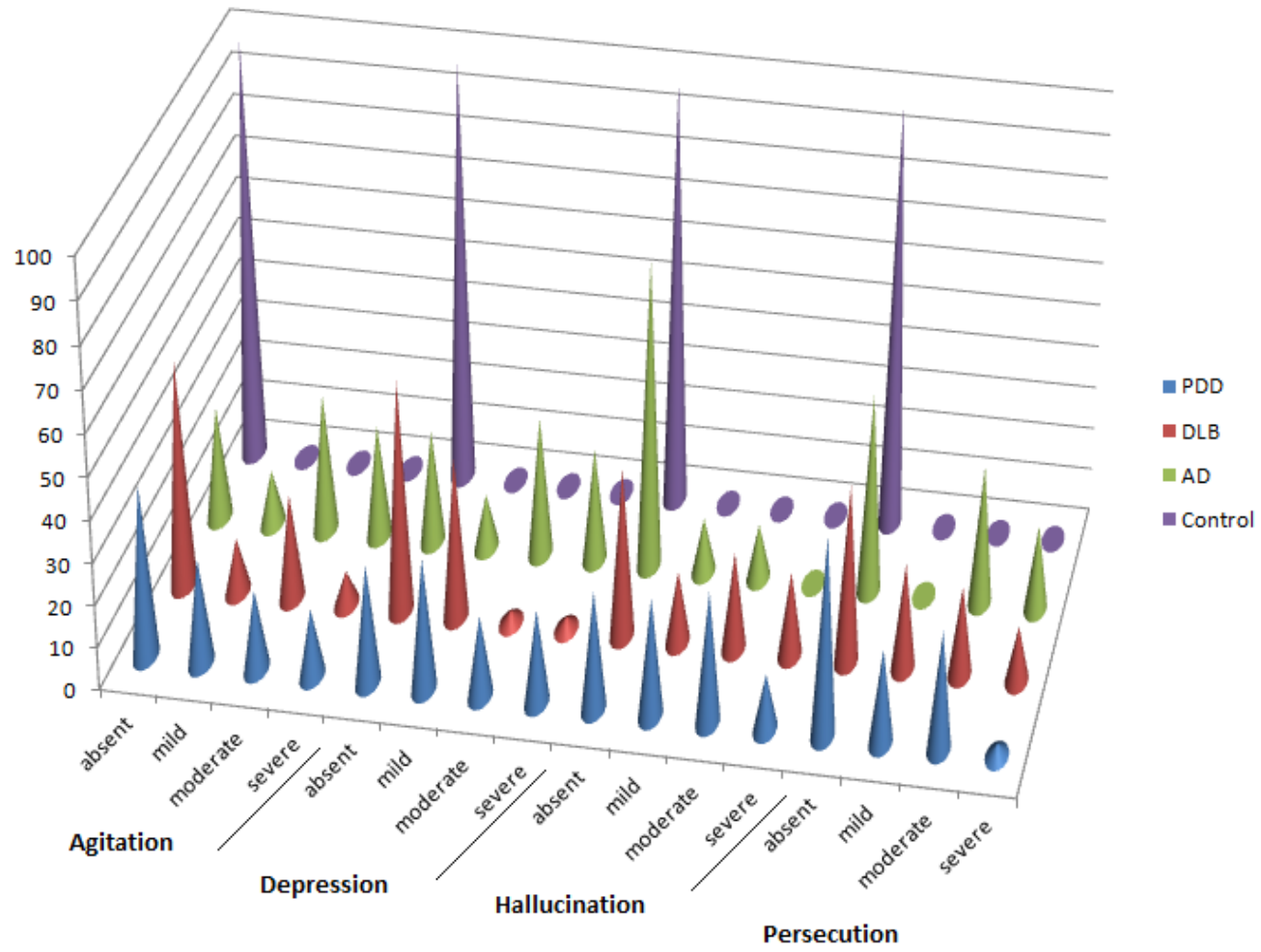


Figure 4.1.5. Frequency of behavioural scores in all cases.

The frequency of each behavioural score was calculated for all cases that had scores and represented graphically, according to clinical diagnosis. The y axis represents the percentage of cases with a score.

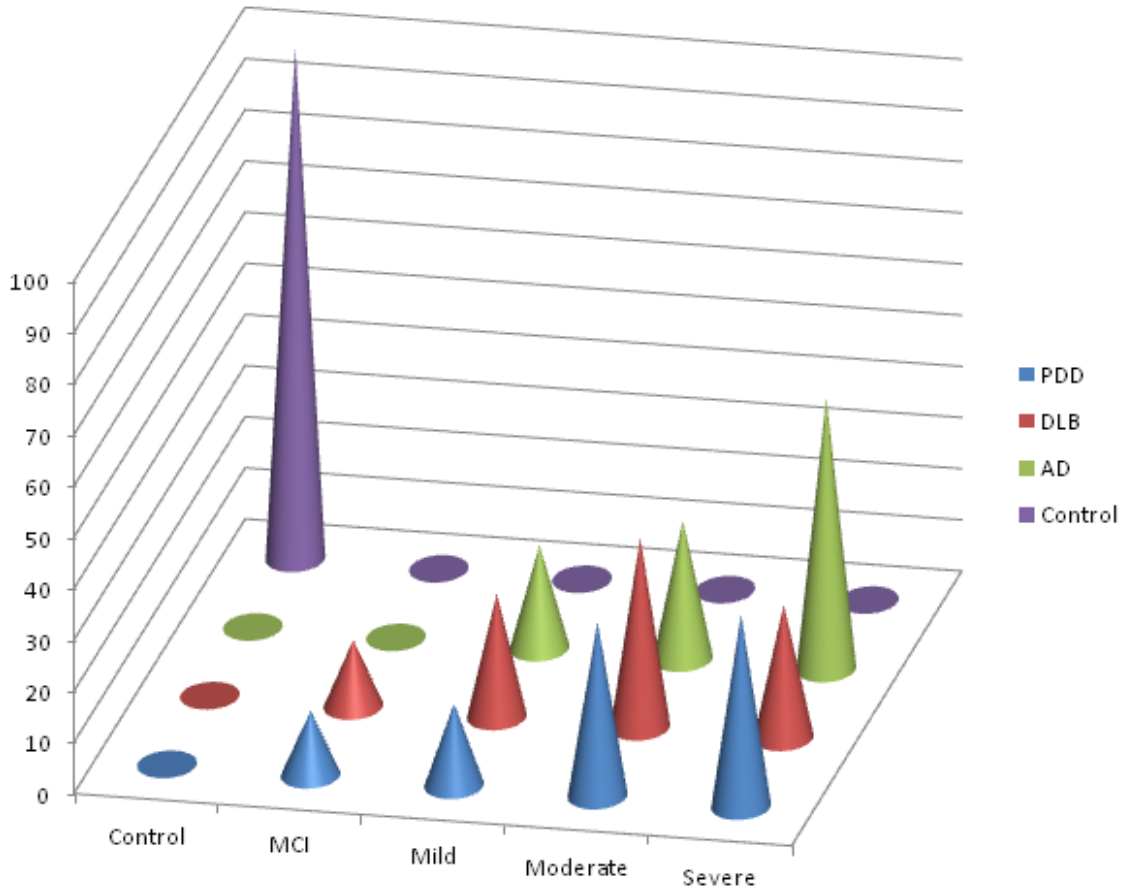


Figure 4.1.6. Percentage of cases in each cognitive impairment category.

The frequency cases in each category of cognitive impairment was calculated based upon MMSE scores (see section 2.1.1 for details) and represented graphically, according to clinical diagnosis (see key on the left of the graph). The y axis represents the percentage of cases within a category.

To determine the relationship of the behavioural symptoms (hallucination, persecution, depression and agitation) to cognition – regression analysis was performed using the scores for these behaviours as independent predictor variables for the category of cognitive impairment cases were classified as falling into (these were 'cognitively unimpaired', 'moderately impaired cognition' and 'severely impaired cognition' – based upon either the final MMSE score prior to death or the selection of a case as a control by the MRC London Neurodegenerative Diseases Brain bank). As a result it was found that both depression and agitation scores significantly predicted cognitive impairment in an inverse manner – the regression values are detailed in figure 4.1.7, part A, and the differences in depression and agitation scores are depicted in the scatter plots in this figure (parts B and C respectively). One-way ANOVA analysis showed cases without cognitive impairment to have significantly lower depression and agitation scores than cases with either moderate or severe cognitive impairment.

The association between pathology and behavioural scores was investigated in the same manner, although Pearson's correlation was used as a screen enabling only those pathology scores that correlated to the behavioural score in question to be entered into the regression analysis as independent variables. This strategy was adopted, in line with advice from a statistician (Stephen Newhouse), due to the large number of different pathology scores and lack of a clear biological rationale behind division of these scores into sub-groups during analysis.

It was found that hallucination scores were predicted by α-synuclein pathology in BA40 in a positive manner (figure 4.1.8). Cases without hallucinations had substantially lower levels of α-synuclein pathology in the parietal cortex than cases with mild, moderate or frequent/severe hallucination scores, illustrated in graph B, figure 4.1.8.

Figure 4.1.7 Depression and agitation scores were significant independent predictors of cognitive impairment (based upon MMSE score).

Multiple regression analysis using the semi-quantitative scores for the four behavioural symptoms of interest (hallucination, persecution, depression and agitation - coded into absent, mild, moderate or severe and assuming control cases to have a score of absent) as independent predictor variables found depression and agitation scores to independently predict the cognitive impairment group that cases were classified as belonging to – based upon MMSE scores. The ANOVA for the model was significant ($p<0.001$). The regression values for depression and agitation were $p=0.03$, Beta = -0.254 and $p=0.042$, Beta = -0.236 respectively. Analysis of the differences in depression and agitation scores between the MMSE groups was performed using non-parametric Mann Whitney-U tests as the homogeneity of variance was significant. Cases in the 'cognitively normal' group were found to have significantly lower depression scores than cases in the 'moderately impaired cognition' and 'severely impaired cognition' groups ($W=879.0$, $Z= -3.833$, $p=0.00$ and $W=780.5$, $Z= -4.441$, $p<0.001$ respectively). Agitation scores were likewise significantly lower in cases belonging to the 'cognitively normal' group than cases in either the 'moderately impaired cognition' or 'severely impaired cognition' ($W=873.5$, $Z= -3.612$, $p<0.001$ and $W=762.5$, $Z= -4.527$, $p<0.001$ respectively). The horizontal bars within the data points represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.532[a]	.283	.251	.70195

ANOVA[b]

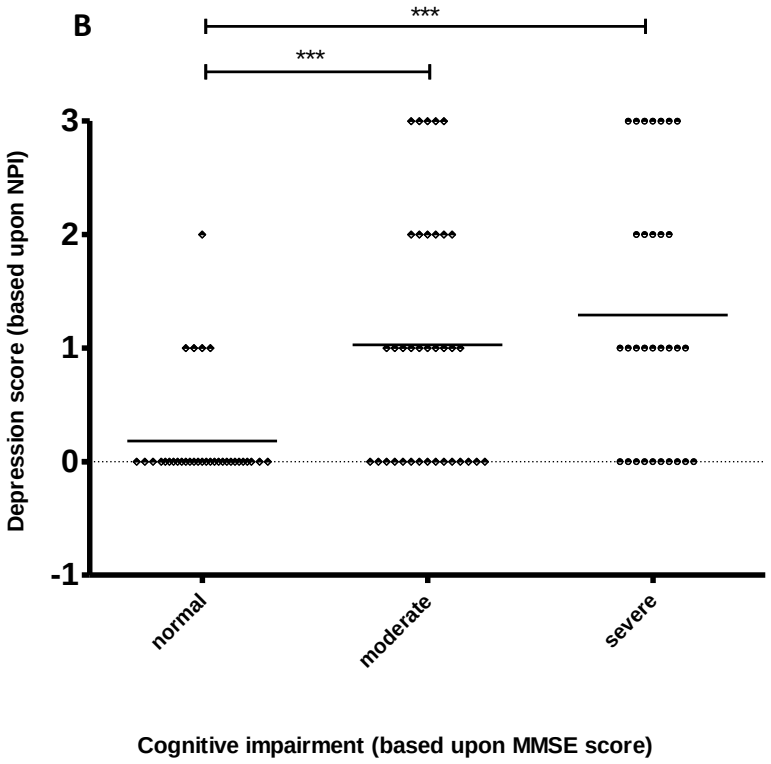
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	17.485	4	4.371	8.871	.000[a]
	Residual	44.347	90	.493		
	Total	61.832	94			

b. Dependent Variable: MMSE prior to death coded into severe, moderate or normal/control

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.468	.104		23.770	.000
	Hallucination score	-.068	.074	-.089	-.909	.366
	Persecution score	-.081	.085	-.101	-.943	.348
	Depression score	-.194	.088	-.254	-2.201	.030
	Agitation score	-.178	.086	-.236	-2.063	.042

a. Dependent Variable: MMSE coded into severe, moderate or normal/control


B
***
***
3
2
1
0
-1
Depression score (based upon NPI)
normal
moderate
severe
Cognitive impairment (based upon MMSE score)

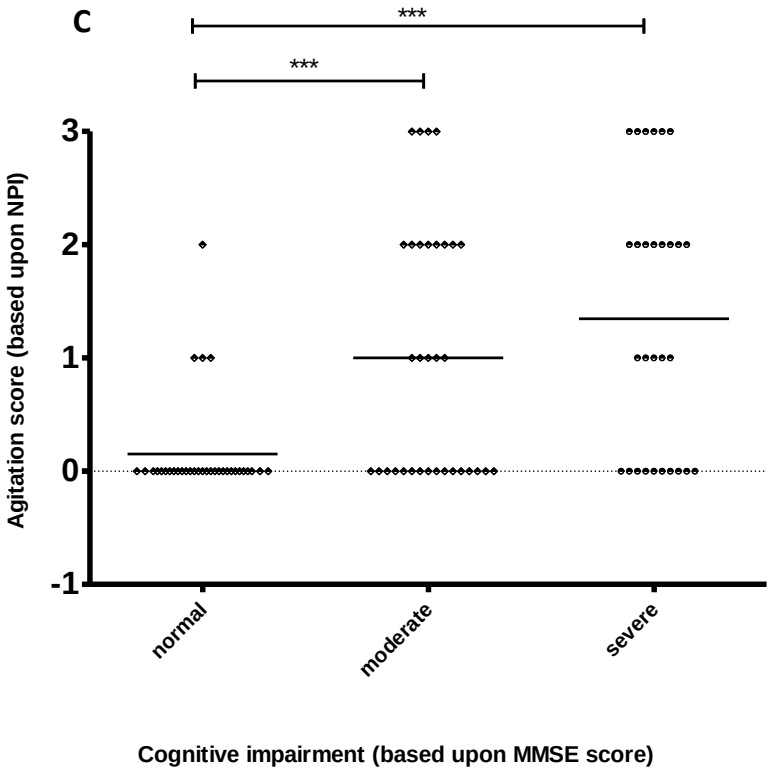

C
***
***
3
2
1
0
-1
Agitation score (based upon NPI)
normal
moderate
severe
Cognitive impairment (based upon MMSE score)

Figure 4.1.8. The α-synuclein score in BA40 predicted hallucination scores.

Regression analysis was utilised to determine if there were significant associations between the semi-quantitative pathology and behavioural scores. Pearson's correlation was used to screen for pathologies that had significant associations to the hallucination score, these were the α-synuclein score in each region and the plaque score in BA24, and were used as independent variables in the regression. It was found that the α-synuclein score in BA40 predicted the hallucination score (image A), when control cases were included (regression values were beta=0.361, p=0.019). The ANOVA for the model was significant (p=0.001). The difference in values for these two pathologies between hallucination score groups was analysed using the Mann-Whitney U test, as the homogeneity of variance was significant. The α-synuclein score in BA40 was significantly lower in cases without hallucinations than cases with mild, moderate or severe hallucinations (graph B; W=1621, Z= -3.093, p=0.002, W=1653.5, Z= -3.449, p=0.001 and W=1474.5, Z= -3.867, p<0.001 respectively). The horizontal lines within data points represent the mean value. *=p<0.05, **=p<0.01 and ***=p<0.001 for all graphs.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.494[a]	.244	.194	.971

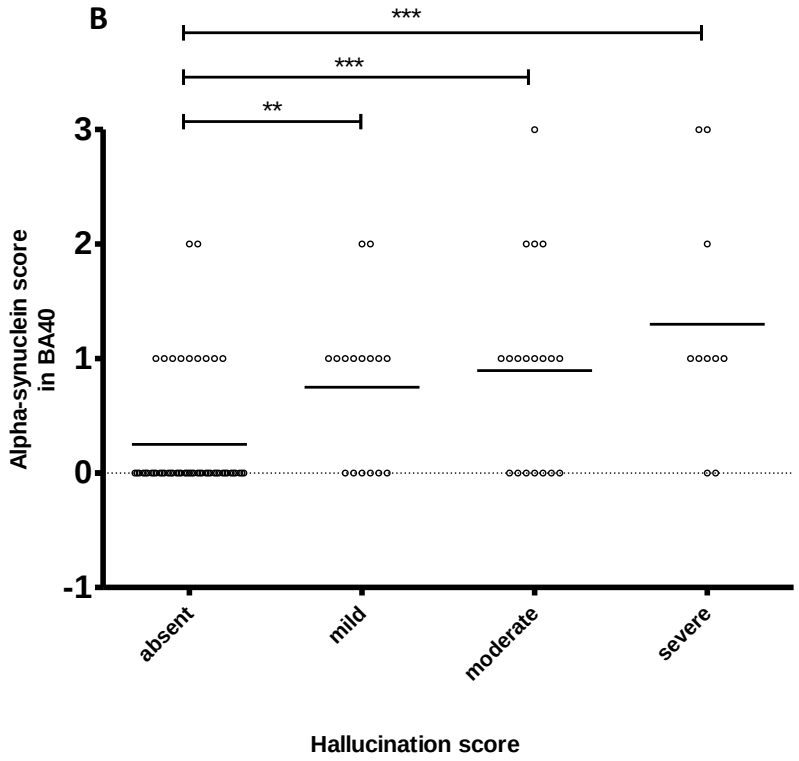
ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	22.829	5	4.566	4.842	.001[a]
	Residual	70.726	75	.943		
	Total	93.556	80			

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.387	.186		2.081	.041
	alpha synuclein score for BA24	.076	.126	.085	.601	.549
	alpha synuclein score for BA21	.078	.192	.067	.408	.684
	alpha synuclein score for BA9	.021	.215	.016	.100	.921
	alpha synuclein score for BA40	.550	.230	.361	2.393	.019
	plaque score for BA24	.066	.107	.065	.617	.539

Dependent Variable: Hallucination score (including controls)


B
***
***
**
3
2
1
0
-1
Alpha-synuclein score in BA40
absent
mild
moderate
severe
Hallucination score

4.2 Analysis of the neuronal and synaptic biochemistry according to clinical diagnosis

4.2.1 Analysis of β-III-tubulin, PSD95, synaptophysin and ZnT3 values in BA9 according to clinical diagnosis

The levels of the four proteins of interest, Btub, PSD95, SPP and ZnT3, in homogenised grey matter from BA9, were determined using semi-quantitative Western blotting. The cases were grouped by clinical diagnosis into control, PDD, DLB and AD to reveal differences in the relative levels of proteins between diagnostic groups, shown in figure 4.2.1. Btub was not significantly altered across any of the diagnostic groups; however, figure 4.2.1 shows PDD cases had significantly lower levels of PSD95, SPP and ZnT3 than control cases. This reduction was also significant compared to DLB and AD cases for PSD95 and SPP, whereas ZnT3 values in DLB cases were significantly lower than controls but not significantly different to PDD. PSD95 values in DLB cases were also significantly lower than controls, yet significantly higher than PDD. Interestingly, the levels of all 4 proteins in AD cases were not significantly different to the control cases.

To determine the effect of AD (Aβ and tau) pathology on protein levels in PDD and DLB, a regression analysis was carried out to examine the effect of AD pathology, and where necessary, to create residual variables for the protein in question that accommodate for any significant predicting of the protein value by either the plaque or tangle scores. As AD pathology did not significantly predict Btub or ZnT3 values this was not done for these proteins.

Figure 4.2.2 shows these 'AD pathology residual' values for PSD95 and SPP, grouped by clinical diagnosis. It can be seen that, despite accounting for the relationship between PSD95 and plaques, and SPP and tangles, there remains a significant reduction in the levels of both proteins in PDD compared to all other diagnostic groups. Additionally, DLB cases continue to have significantly lower PSD95 levels than control cases.

Figure 4.2.1 Protein values, from semi-quantitative Western blotting in BA9, by diagnosis.

Statistical analysis was performed using One-way ANOVA and Bonferroni post-hoc test. The ANOVA for Btub values (graph A) was significant but there was no difference between groups according to the post hoc test. ZnT3 values (graph B) for the control group were significantly higher than the PDD ($p<0.001$) and DLB ($p=0.001$) groups. PSD95 values (graph B) for the control group were significantly higher than the PDD ($p<0.001$) and DLB ($p=0.001$) groups. PSD95 values for the PDD group were significantly lower than the AD ($p=0.012$) and DLB ($p=0.036$) groups. SPP values (graph C) for the PDD group were significantly lower than the control ($p=0.003$), DLB ($p<0.001$) and AD ($p<0.001$) groups. The AD group SPP values were significantly higher than the control group ($p=0.005$) and DLB group ($p=0.009$). The ANOVA values are as follows; Btub, $F=2.819$, $df=3,111$, PSD95; $F=12.809$, $df=3,108$, $p<0.001$, SPP; $F=4.862$, $df=3,97$, $p=0.003$ and ZnT3; $F=9.409$, $df=3,111$, $p<0.001$. The horizontal bars within the data points in the graphs represent the mean values.

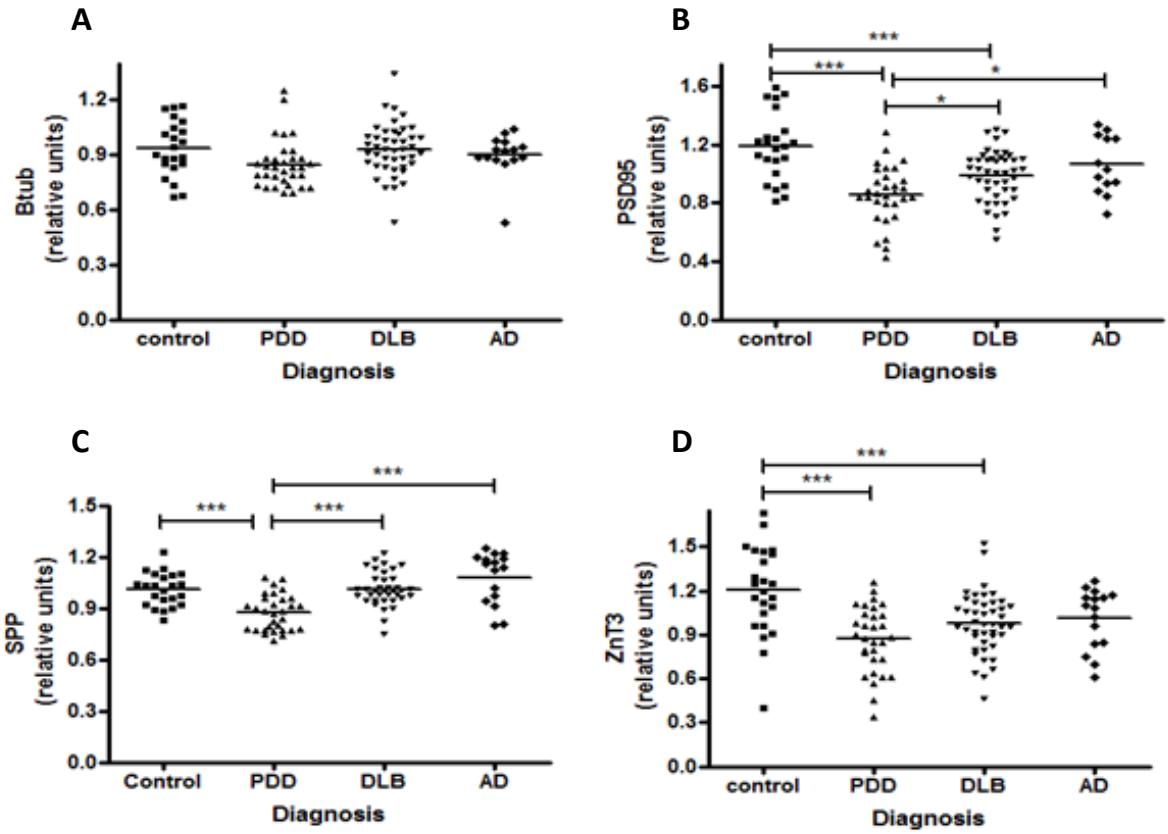
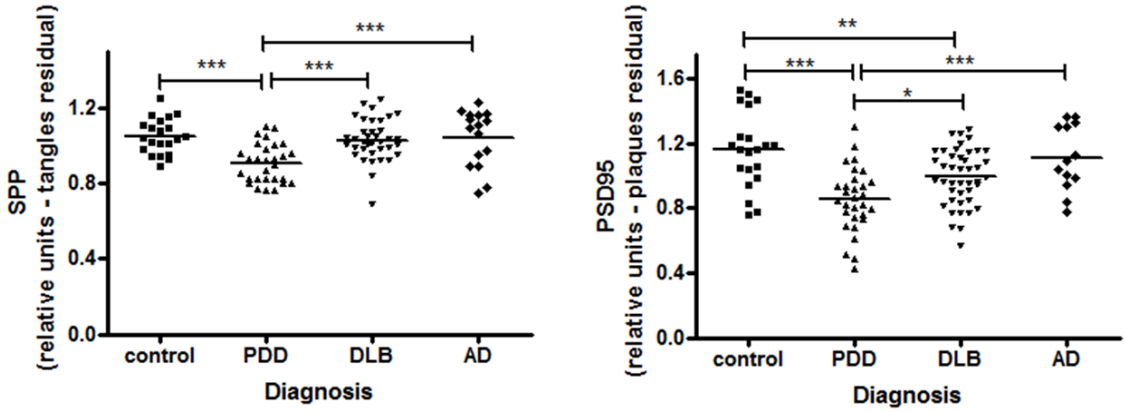


Figure 4.2.2 SPP and PSD95 protein values, from semi-quantitative Western blotting in BA9, expressed as residuals for tangle and plaque scores respectively, and grouped by diagnosis.

Statistical analysis was performed using One-way ANOVA and Bonferroni post-hoc test. SPP tangles residual values were significantly lower in the PDD group compared to the control group (p=0.002), DLB group (p=0.002) and AD group (p=0.001). For the ANOVA; F=9.691, df= 3,99, p<0.001. PSD95 plaque residual values were significantly lower in the PDD group compared to the control group (p<0.001), the DLB group (p=0.02) and AD group (p=0.001). Control values were significantly higher than DLB values (p=0.01). For the ANOVA; F=11.870, df=3,103, p<0.001. There was no significant difference between AD and DLB or control values for either protein. The horizontal bars within the data points on the graphs represent the mean values.



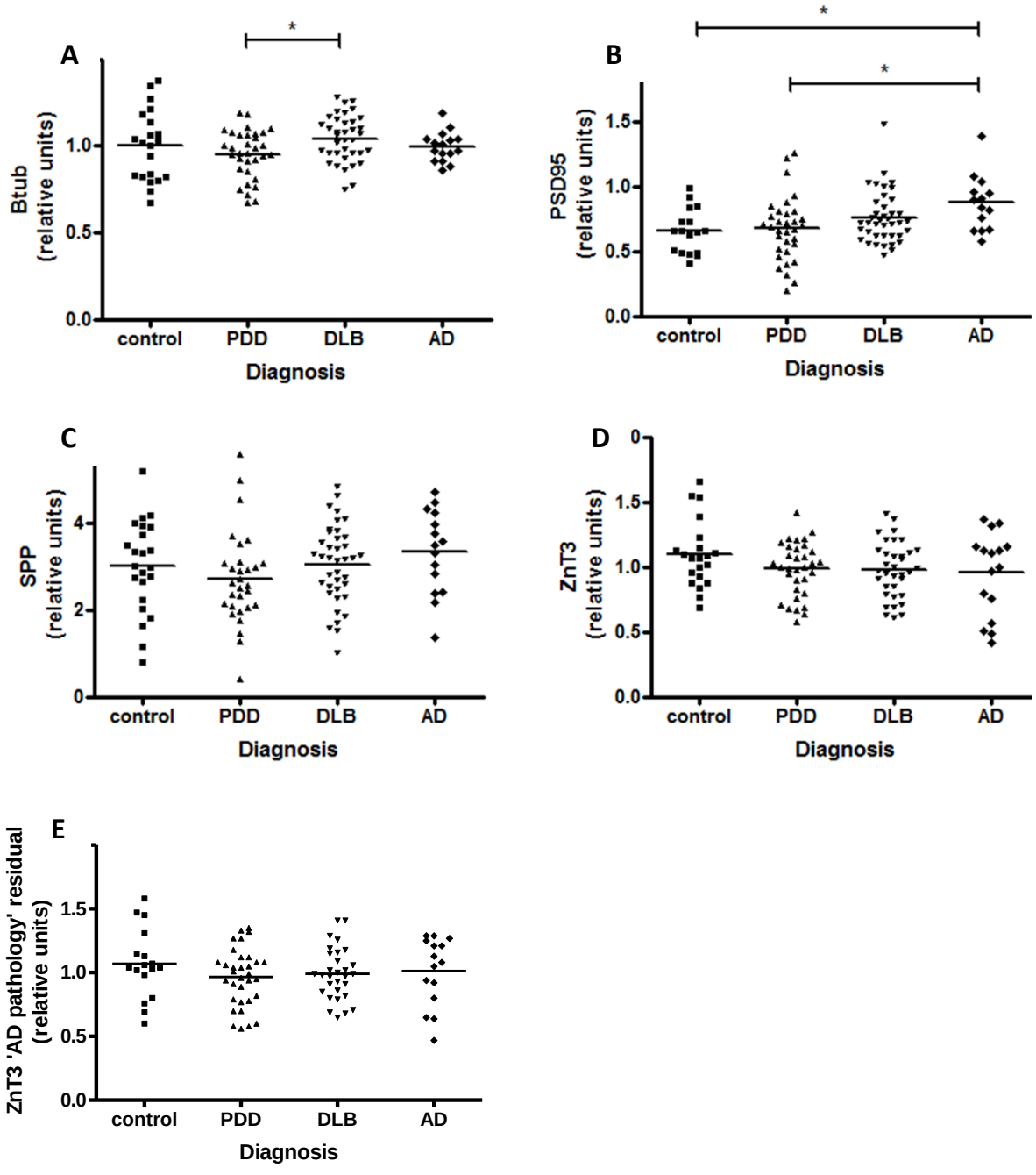
4.2.2 Analysis of β-III-tubulin, PSD95, synaptophysin and ZnT3 values in BA24 according to clinical diagnosis

The levels of the four proteins of interest, Btub, PSD95, SPP and ZnT3, in homogenised grey matter from BA24, were determined using semi-quantitative Western blotting. The cases were grouped by clinical diagnosis into control, PDD, DLB and AD to reveal differences in the relative levels of proteins between diagnostic groups, shown in figure 4.2.3. The only proteins with significant changes in value between any of the diagnostic groups were PSD95 and Btub – the former was significantly higher in AD cases compared to control and PDD cases, and the latter significantly lower in PDD versus DLB. The distribution of some protein values was characterised by a high variance – in particular SPP values. Contrastingly, Btub values were particularly clustered.

To determine the effect of AD (Aβ and tau) pathology on protein levels in PDD and DLB, a regression analysis was carried out to examine the effect of AD pathology, and where necessary, to create residual variables for the protein in question that accommodate for any significant predicting of the protein value by either the plaque or tangle scores. However, AD pathology (plaques and tangles) was only a significant predictor of ZnT3. The ZnT3 'AD pathology' residual is shown in the bottom graph in figure 4.2.2 – where it can be seen that there was no significant difference in ZnT3 values between diagnostic groups after removing the effect of Ad pathology in ZnT3 levels in BA24.

Figure 4.2.3 – Proteins values, obtained from semi-quantitative Western blotting in BA24, by clinical diagnosis

Statistical analysis (One-way ANOVA and Bonferroni post hoc test) confirmed there was no significant difference between diagnostic groups for SPP and ZnT3 values (graphs C and D) but showed PSD95 values were significantly higher in AD cases compared to control (p=0.037) and PDD cases (p=0.027) (graph B). For the ANOVA (PSD95); F=3.745, df=3,100, p=0.013. Btub values were analysed non-parametrically using Mann Whitney-U tests, (as the homogeneity of variance was significant) which revealed DLB values to be significantly higher than PDD (W=965.0, Z= -2.43, p=0.015) (graph A). There was no significant difference between diagnostic groups for the ZnT3 'AD pathology' residual variable (graph E). The horizontal bars within the data points on the graphs represent the mean values.



4.2.3 Analysis of β-III-tubulin, PSD95, synaptophysin and ZnT3 values in BA40 according to clinical diagnosis

The levels of the four proteins of interest, Btub, PSD95, SPP and ZnT3, in homogenised grey matter from BA40, were determined using semi-quantitative Western blotting. The cases were grouped by clinical diagnosis into control, PDD, DLB and AD to reveal differences in the relative levels of proteins between diagnostic groups, shown in figure 4.2.4. AD cases were characterised by significant reductions, compared to all other diagnostic groups, for the synaptic and neuronal marker proteins PSD95, SPP and Btub - excepting PSD95, which was unchanged between DLB and AD cases. ZnT3 values were not significantly different between any diagnostic groups.

To determine the effect of AD (Aβ and tau) pathology on protein levels in PDD and DLB, a regression analysis was carried out to examine the effect of AD pathology, and where necessary, to create residual variables for the protein in question that accommodate for any significant predicting of the protein value by either the plaque or tangle scores. Tangle scores significantly predicted all four proteins of interest in BA40 and so residual variables were created to account for this effect (see methods section for details). Figure 4.2.5 shows that there was no significant difference in the levels of any of the proteins after the effect of tangles on protein values was removed.

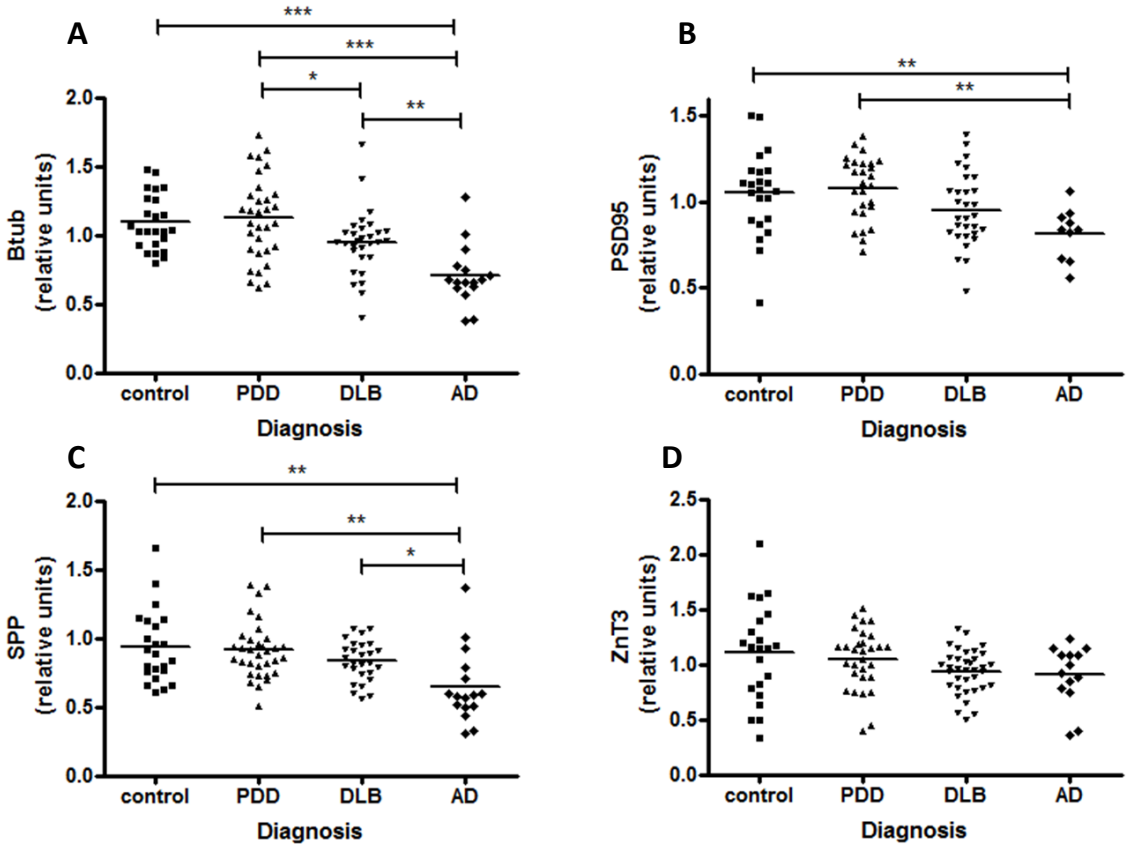


Figure 4.2.4 – Btub, PSD95, SPP and ZnT3 values, obtained from semi-quantitative Western blotting in BA40, by clinical diagnosis

Statistical analysis was performed using One-way ANOVA and Bonferroni post-hoc test. Graph A shows AD Btub values were significantly lower than all other groups (PDD and control $p<0.001$, DLB $p=0.017$); DLB Btub values were significantly lower than PDD ($p=0.04$). AD PSD95 values (graph B) were significantly lower than control ($p=0.02$) and PDD ($p=0.005$). AD SPP values (graph C) were significantly lower than all other diagnostic groups (control and PDD $p=0.001$, DLB $p=0.049$). There was no significant difference in ZnT3 values between diagnostic groups (graph D). The ANOVA values were; Btub, $F=11.395$, $df=3,98$, $p<0.001$, PSD95, $F=5.04$, $df=3,86$, $p=0.003$, SPP, $F=6.891$, $df=3,95$, $p<0.001$. The horizontal bars within the data points represent the mean values.

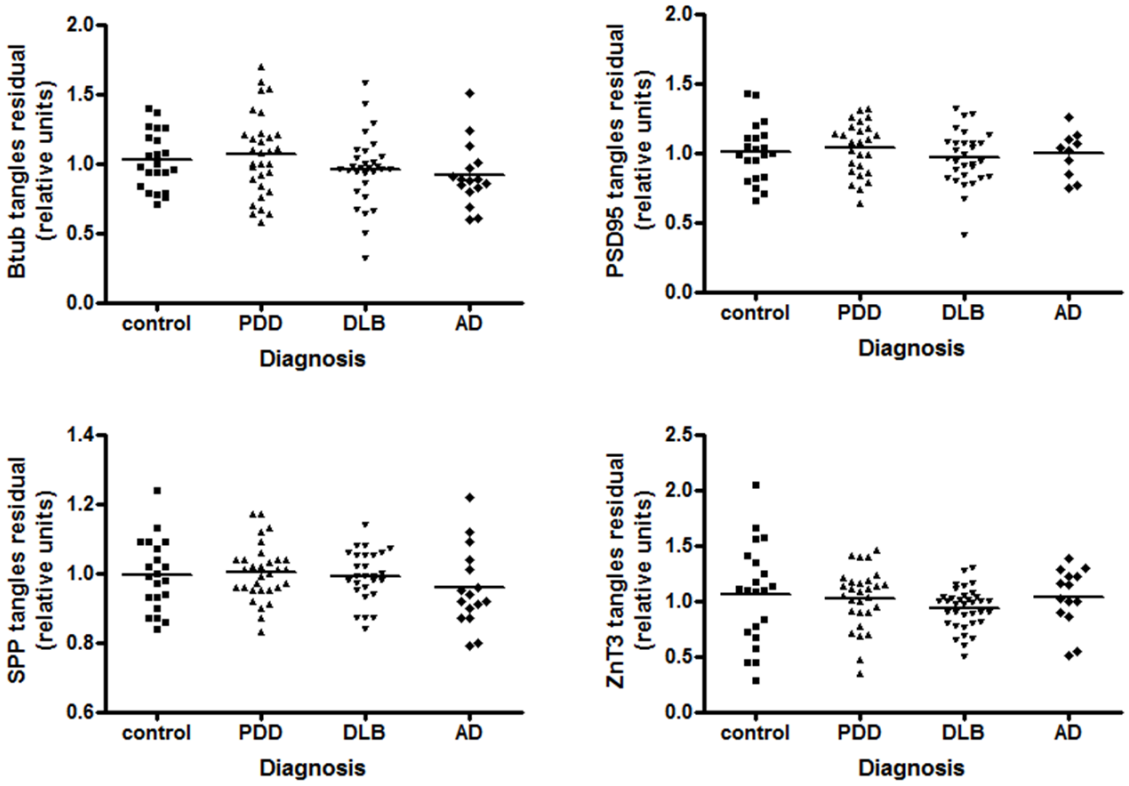


Figure 4.2.5 Btub, PSD95, SPP and ZnT3 values, from semi-quantitative Western blotting in BA40, expressed as residuals for tangle scores, and grouped by diagnosis.

The semi-quantitative score for tangles correlated to the values for all four proteins in BA40 and significantly predicted the values of each protein. To counter this effect of tangles on the protein values residual variables for each protein were created through linear regression analysis and normalised by transformation when necessary. Statistical analysis was performed using One-way ANOVA and Bonferroni post-hoc test and found no significant difference between any diagnostic groups for any of the proteins. The horizontal bars within the data points of the graphs represent the mean values.

Analysis of the ratio of the pre and post-synaptic markers SPP and PSD95 to the neuronal marker Btub, and of ZnT3 to the pre-synaptic marker SPP, in BA9, according to clinical diagnosis.

The values obtained from quantification of the Western blots were used to create ratios to determine the relative changes in pre and post-synaptic terminals over and above any change in the neuronal marker Btub, and to determine the change in ZnT3 levels over and above any change in the levels of pre-synaptic terminals. The differences in these ratios in BA9 between diagnostic groups are shown in figure 4.2.6. The ratio of SPP to Btub was similar between diagnostic groups – apart from a significant increase in AD cases compared to PDD cases. The ratio of PSD95 to Btub was significantly lower in PDD and DLB cases verses controls, and unchanged in AD cases. However, the ratio of ZnT3 to SPP was characterised by significant reductions in all three dementias compared to controls – quite substantial in the case of DLB and AD ($p=0.001$) – which were also reduced relative to PDD cases.

To determine the effect of AD pathology on these ratios a regression analysis was carried out to establish whether plaque or tangle scores significantly predicted any of the ratios, and if so, to create residual variables for the ratios in question that accommodate for the significant predicting of the ratio value by either the plaque or tangle scores. This was carried out for the ratio of ZnT3 to SPP, - with respect to the tangle score, shown in the bottom-right graph in figure 4.2.6. When the effect of the tangle score was thus statistically compensated, there was no significant difference in the ratio of ZnT3 to SPP between diagnostic groups.

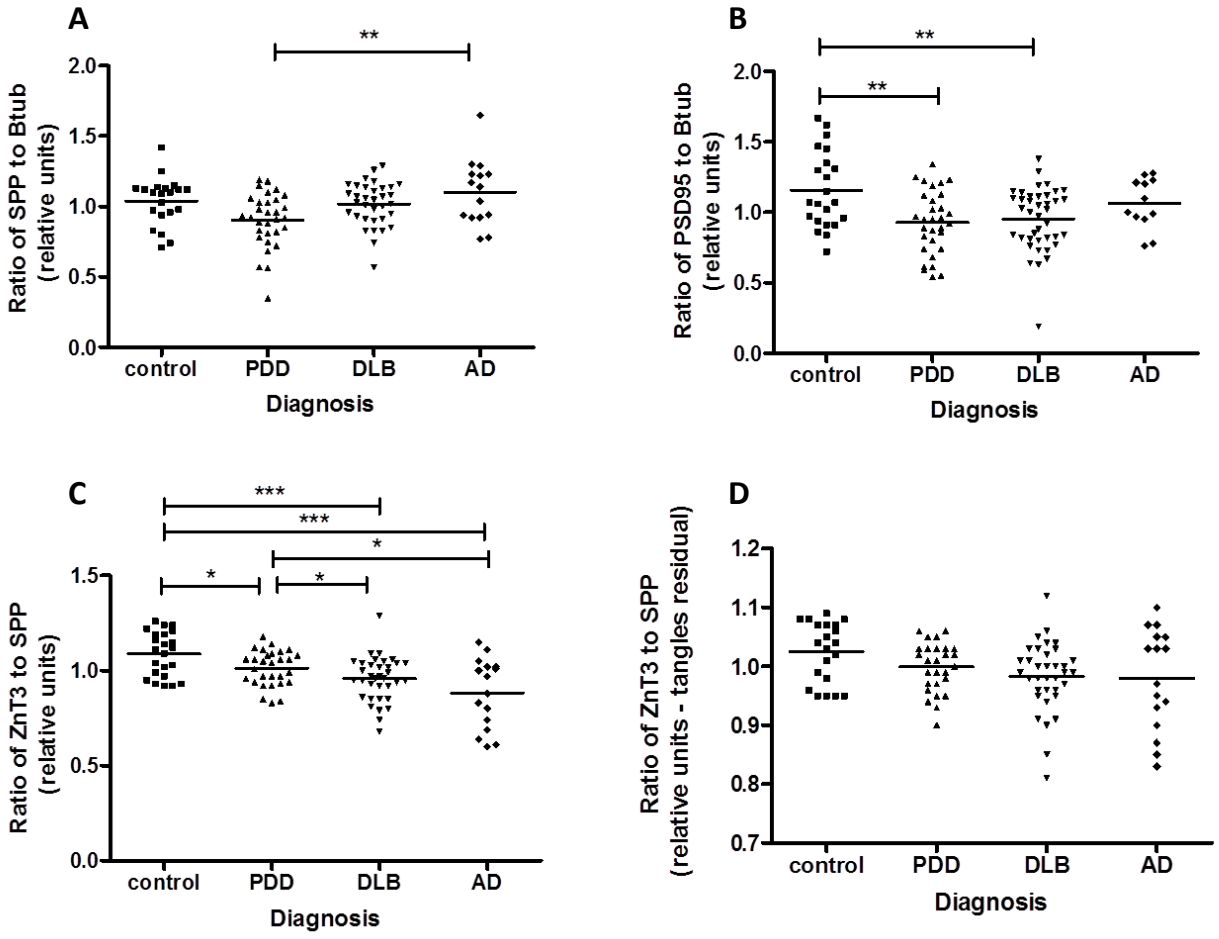


Figure 4.2.6 Ratio of SPP to Btub, PSD95 to Btub and ZnT3 to SPP in BA9 grouped by diagnosis.

Analysis was carried out using one-way ANOVA and Bonferroni post-hoc test. The ratio of SPP to Btub (graph A) was significantly higher in AD cases compared to PDD cases (p=0.005). The ANOVA values were; F=4.862, df=3,97, p=0.003. The ratio of PSD95 to Btub (graph B) was significantly higher in control cases compared to PDD (p=0.004) and DLB (p=0.007) cases. ANOVA values were; F=5.23, df=3,101, p=0.002. The homogeneity of variance for the ratio of ZnT3 to SPP was significant and so non-parametric analysis (Mann Whitney-U) was used and showed significantly higher values in control cases compared to PDD (W=650.0, Z= -2.183, p=0.029), DLB (W820.0, Z= -3.378, =p=0.001) and AD cases (W=207.0, Z= -3.226, p=0.001), and in PDD cases compared to DLB (w=972.0, Z= -2.232, p=0.026) and AD cases (W=273.0, Z= -2.253, p=0.024) (graph C). There was no significant difference between diagnostic groups for the 'tangle residual' ZnT3 to SPP ratio (graph D). Horizontal bars within the data points represent mean values.

4.2.4 Analysis of the ratio of the pre and post-synaptic markers SPP and PSD95 to the neuronal marker Btub, and of ZnT3 to the pre-synaptic marker SPP, in BA24, according to clinical diagnosis.

The values obtained from quantification of the Western blots were used to create ratios to determine the relative changes in pre and post-synaptic terminals over and above any change in the neuronal marker Btub, and to determine the change in ZnT3 levels over and above any change in the levels of pre-synaptic terminals. The differences in these ratios in BA24 between diagnostic groups are shown in figure 4.2.7. The only significant change for any of the ratios was that of PSD95 to Btub in AD cases – which were increased in comparison to the other diagnostic groups. There was no significant relationship between AD pathology in BA24 and these ratios and so no further investigation was undertaken into any effect AD pathology may have on the ratios.

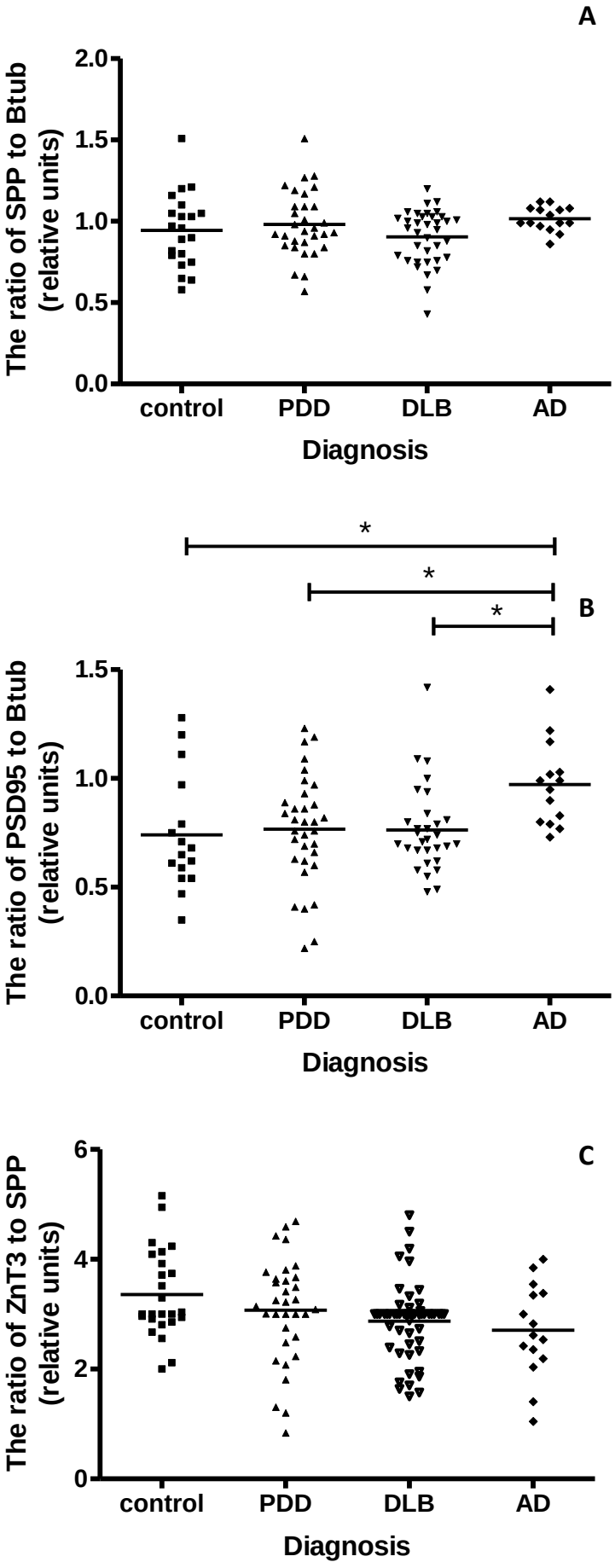


Figure 4.2.7 The ratios of SPP to Btub, PSD95 to Btub and ZnT3 to SPP by diagnostic group in BA24

Analysis of the difference between the protein ratios across diagnostic groups was performed using one-way ANOVA and Bonferroni post-hoc tests when the test for homogeneity of variance was met – which was the case for the ratio of PSD95 to Btub and the ratio of ZnT3 to SPP. The homogeneity of variance was significant for the ratio of SPP to Btub and so nonparametric analysis was used, which showed no significant difference between diagnostic groups (graph A). The ratio of PSD95 to Btub was significantly higher in AD cases compared to control (p=0.043), PDD (p=0.037) and DLB (p=0.035) cases (graph B). The ANOVA values were; F=3.437, df=3,89, p=0.02. Whilst the ANOVA for the ratio of ZnT3 to SPP was significant (F=2.928, df=3,124 p=0.036) there was no significant difference between diagnostic groups according to the Bonferroni post-hoc test (graph C).

4.2.5 Analysis of the ratio of the pre and post-synaptic markers SPP and PSD95 to the neuronal marker Btub, and of ZnT3 to the pre-synaptic marker SPP, in BA40, according to clinical diagnosis.

The values obtained from quantification of the Western blots were used to create ratios to determine the relative changes in pre and post-synaptic terminals over and above any change in the neuronal marker Btub, and to determine the change in ZnT3 levels over and above any change in the levels of pre-synaptic terminals. The differences in these ratios in BA40 between diagnostic groups are shown in figure 4.2.8. There was no significant difference in the ratios of SPP or PSD95 to Btub between diagnostic groups; however, the ratio of ZnT3 to SPP was significantly lower in DLB cases than all other cases. There was no significant relationship between AD pathology in BA40 and the ratios described above, and so no further investigation was undertaken into any effect AD pathology may have on the ratios.

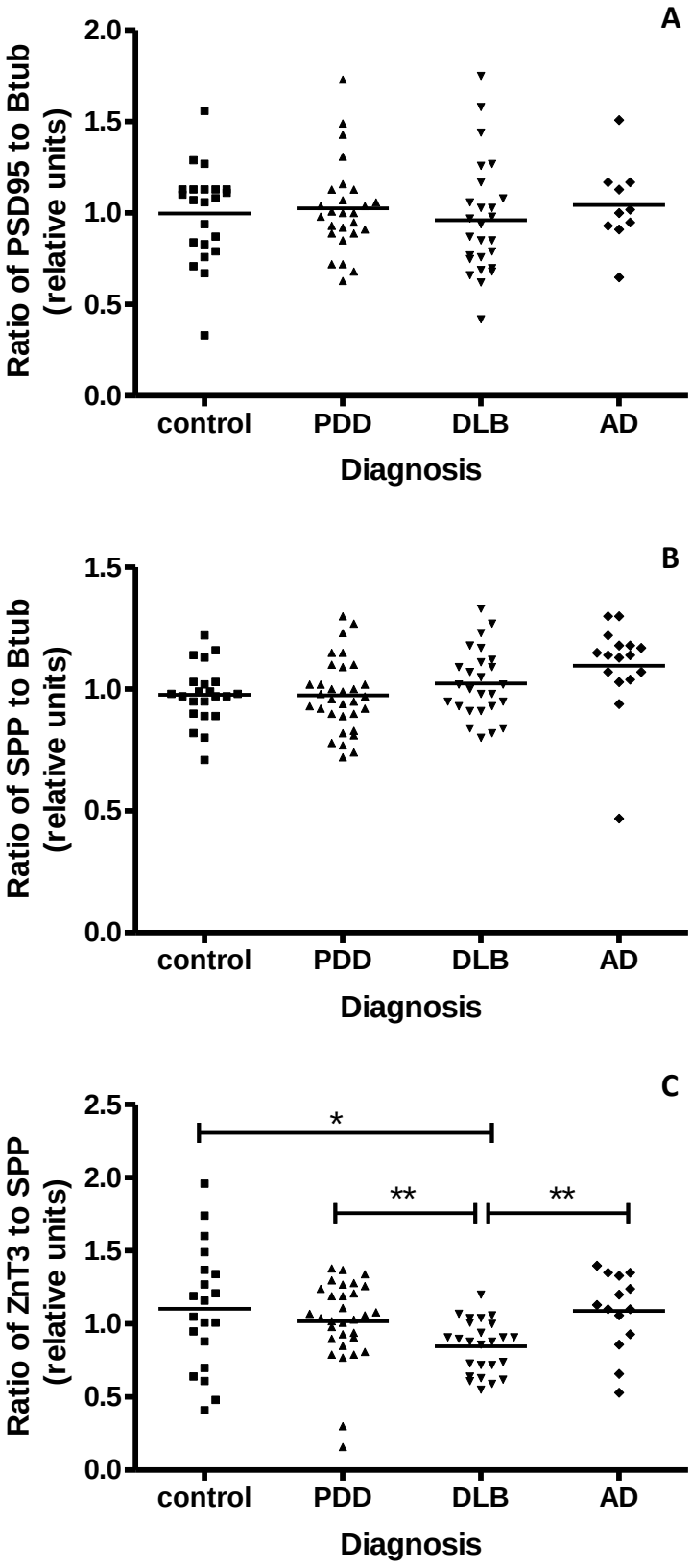


Figure 4.2.8 Ratio of SPP to Btub, PSD95 to Btub and ZnT3 to SPP values, derived from semi-quantitative western blotting in BA40, and grouped by diagnosis.

Analysis was carried out using one-way ANOVA and Bonferroni post-hoc test for SPP to Btub and PSD95 to Btub and revealed no significant difference between diagnostic groups (graphs A and B respectively), although the ANOVA was significant for the ratio of SPP to Btub ($F=2.854$, $df=3,86$, $p=0.041$). The homogeneity of variance was significant for the ratio of ZnT3 to SPP and so the non-parametric Mann-Whitney U test was used for analysis, and showed the ratio of ZnT3 to SPP (graph C) was significantly lower in DLB cases compared to controls ($W=472.0$, $Z= -2.353$, $p=0.019$) and AD ($W=397.0$, $Z= -3.016$, $p=0.002$) cases and PDD cases ($W=521.5$, $Z= -3.148$, $p=0.002$). Horizontal bars within the data points represent mean values.

4.3 Relationships between clinical data and synaptic and neuronal biochemistry from semi-quantitative Western blotting

Regression analysis was undertaken to investigate the relationship between the proteins of interest and the behavioural symptom scores. Each brain region was analysed separately to avoid contrasting patterns of increased or decreased levels for the same protein in different regions masking an effect on a symptom, and due to biological rationale linking some symptoms to a brain region (see discussion).

When regression analysis was performed using the values from semi-quantitative Western blotting (after statistical processing) for Btub, PSD95, SPP and ZnT3 in BA9 as independent predictor variables for the depression score, including control cases under the assumption they did not have depression, it was found that ZnT3 values in BA9 significantly predicted the severity of depression. This predictive relationship was inverse, indicated by the negative beta value, such that a decrease in ZnT3 value related to an increase in incident of depression. The output from the regression analysis and a graphic depiction of the relationship between ZnT3 in BA9 and depression score is shown in figure 4.3.1. The scatter plot highlights the difference in ZnT3 values between the depression score groups, which was significantly higher in cases with no depression than cases with a score of severe.

Regression analysis using values from semi-quantitative Western blotting (after statistical processing) for Btub, PSD95, SPP and ZnT3 in BA40 as independent predictor variables for the hallucination score established the SPP value to be a significant predictor, in a direct manner, indicated by the positive beta value. Thus, an increase in SPP value related to an increase in the incident of hallucinations. Control cases were excluded from this analysis as they were characterised by low hallucination scores and high SPP values, relative to dementia cases, and therefore masked the relationship between hallucinations and SPP observed in dementia cases. Figure 4.3.2 shows the values from the regression analysis and a scatter plot depicting the SPP values by hallucination score

– cases with the highest hallucination score had significantly higher SPP values than cases without hallucinations.

Figure 4.3.1 ZnT3 levels, obtained from semi-quantitative Western blotting in BA9, predict depression.

Depression in dementia (DLB, PDD and AD) cases and control cases was significantly predicted by ZnT3 values from semi-quantitative Western blotting in BA9. (Beta= -0.358, p=0.002). Analysis was performed using multiple regression with SPP, PSD95, Btub and ZnT3 as independent predictor variables, according to the analysis strategy. The ANOVA for the model was significant (p=0.001, Rsq= 0.207) suggesting that the proteins have a combined predictive effect on depression scores. One-way ANOVA and Bonferroni post-hoc analysis showed ZnT3 levels to be significantly higher in cases without depression compared to cases with severe depression (p=0.018). The ANOVA values were; F=4.534, df=3,91, p=0.005). The horizontal bars within the data points represent the mean.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.455[a]	.207	.165	.945

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	17.474	4	4.368	4.889	.001[b]
	Residual	67.014	75	.894		
	Total	84.487	79			

a. Dependent Variable: Depression score (including control cases)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.089	.755		2.766	.007
	Btub (relative units)	-1.421	.817	-.185	-1.739	.086
	ZnT3 (relative units)	-1.355	.423	-.358	-3.203	.002
	PSD95 (relative units)	-.682	.482	-.154	-1.414	.161
	SPP (relative units)	.773	.879	.098	.879	.382

a. Dependent Variable: Depression score (including control cases)

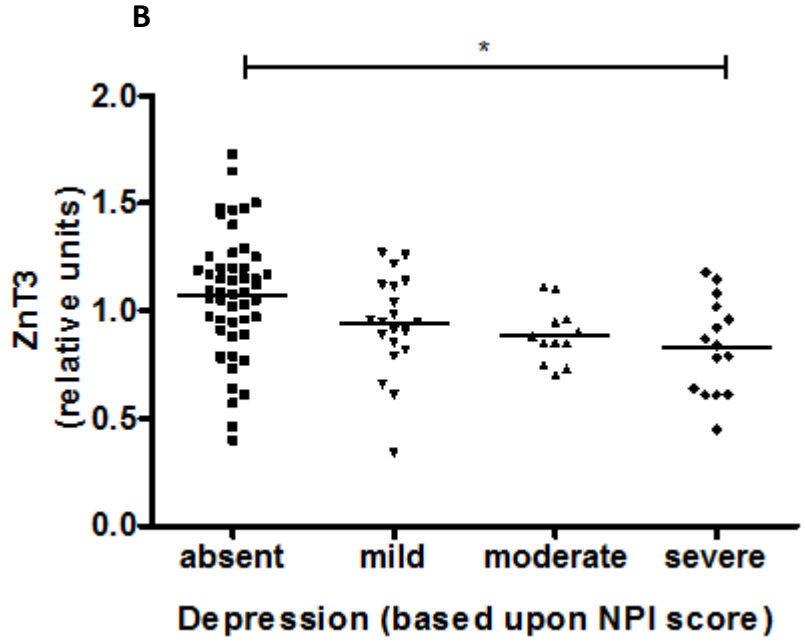

B
*
2.0
1.5
1.0
0.5
0.0
ZnT3
(relative units)
absent
mild
moderate
severe
Depression (based upon NPI score)

Figure 4.3.2 SPP levels in BA40, from semi-quantitative Western blotting, predict hallucinations within dementia cases.

Multiple regression analysis (with Btub, PSD95, SPP and ZnT3 values from semi-quantitative Western blotting as independent variables/predictors) showed that hallucinations in dementia cases only (DLB, PDD and AD) were significantly predicted by SPP values in BA40 (Beta=0.475, p=0.005). Analysis was performed using multiple regression with SPP, PSD95, Btub and ZnT3 as independent predictor variables, according to the analysis strategy. The ANOVA for the model was significant (p=0.031, Rsq=0.191) suggesting that the proteins have a combined predictive effect on hallucination scores. One-way ANOVA and Bonferroni post-hoc analysis showed SPP levels to be significantly higher in cases with severe hallucinations compared to cases without hallucinations (a score of absent) p=0.017; ANOVA: F=3.479, (df=3,68), p=0.021. The horizontal bars within the data points represent the mean.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.437[a]	.191	.125	1.050

ANOVA[a]

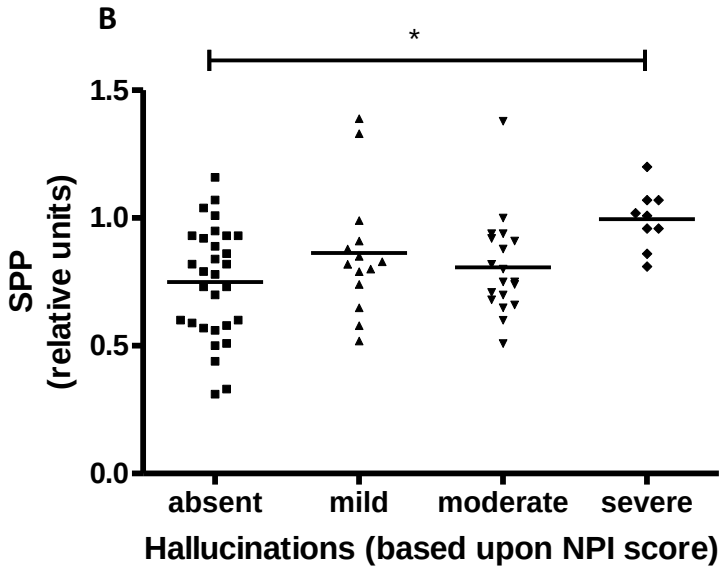
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	12.767	4	3.192	2.894	.031[b]
	Residual	54.048	49	1.103		
	Total	66.815	53			

a. Dependent Variable: Hallucination (NPI coded; dementia cases only)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.913	.724		-1.261	.213
	SPP (relative units)	2.489	.841	.475	2.959	.005
	PSD95 (relative units)	.295	.884	.056	.334	.740
	Btub (relative units)	-.645	.561	-.183	-1.151	.255
	ZnT3 (relative units)	.268	.746	.056	.359	.721

a. Dependent Variable: Hallucination (NPI coded; dementia cases only)


B
*
1.5
1.0
0.5
0.0
SPP
(relative units)
absent
mild
moderate
severe
Hallucinations (based upon NPI score)

Cases were classified into groups according to the degree of cognitive impairment using either the final MMSE score prior to death, or acceptance by the MRC London Neurodegenerative Diseases Brain bank as a control case. The groups were; control cases, cognitively normal/MCI (MMSE of 25 to 30 but diagnoses as dementia), dementia with mild cognitive impairment (as opposed to MCI, MMSE of 17 to 24), moderately impaired cognition (MMSE of 10 to 16) and severely impaired cognition (MMSE of 9 or less), (see the methods section for details about the creation of these groups). It was found, using regression analysis, that PSD95 and ZnT3 values in BA9 significantly predicted cognitive impairment, in an inverse manner. This is illustrated in figure 4.3.3. The ratios of PSD95 to Btub and of ZnT3 to SPP in BA9 were also significant predictors of cognitive impairment, shown in figure 4.3.4. The distribution, between cognitive impairment groups, of all proteins of interest and protein ratios, in each brain region, was analysed by one-way ANOVA and Bonferroni post-hoc test. Scatter plots for the proteins that had significantly different levels between 'cognitive impairment' groups are shown in figure 4.3.5. SPP values in BA40 were significantly lower in cases classified as having severely impaired cognition compared to cases without cognitive impairment, but not significantly different for cases classified as having moderate cognitive impairment to either of the other groups. ZnT3 and PSD95 values in BA9 were significantly lower in cases classified as having severe cognitive impairment and moderate cognitive impairment compared to cases without cognitive impairment. Figure 4.3.3 shows the values from the regression analysis and a scatter plot depicting the ZNT3 to SPP ratio by MMSE group – cases with MMSE scores meriting classification as 'severe cognitive impairment' had significantly lower ratios of ZnT3 to SPP than cases without cognitive impairment.

Figure 4.3.3. PSD95 and ZnT3 values in BA9 predict disease severity based upon the classification of cognitive impairment.

Regression analysis using the values for Btub, PSD95, SPP and ZnT3 from BA9 as independent predictor variables showed PSD95 and ZnT3 values to be significant predictors of the cognitive impairment group, which is a marker for disease severity (beta= -0.246, p=0.021 and beta= -0.377, p=0.001 respectively). The ANOVA for the model was significant (p<0.001). The cognitive impairment groups were; 'controls' (assigned a value of 1 and including all designated control cases), 'MCI/normal' (dementia cases assigned a value of 2 and based on a MMSE score of 25 to 30), 'mild dementia' (assigned a value of 3 and based upon a MMSE score of 17 to 24), 'moderate dementia' (assigned a value of 4 and based upon a MMSE score of 10 to 16) and 'severe dementia' (assigned a value of 5 and based upon a MMSE score of 9 or less). The difference in PSD95 and ZnT3 values between cognitive impairment groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed ZnT3 values to be significantly higher in controls compared to cases with severe dementia (p=0.003), moderate dementia (p=0.001), mild dementia (p=0.021) and MCI/normal cognition (p=0.044). PSD95 values were calculated to be significantly higher in control cases than in cases with moderate dementia (p<0.001) and severe dementia (p=0.003). The ANOVA values were; F=6.043, df=4,90, p<0.001 for PSD95 and F=5.731, df=4,95, p<0.001 for ZnT3. The horizontal bars within the data points in the graphs represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.516[a]	.266	.228	1.35330

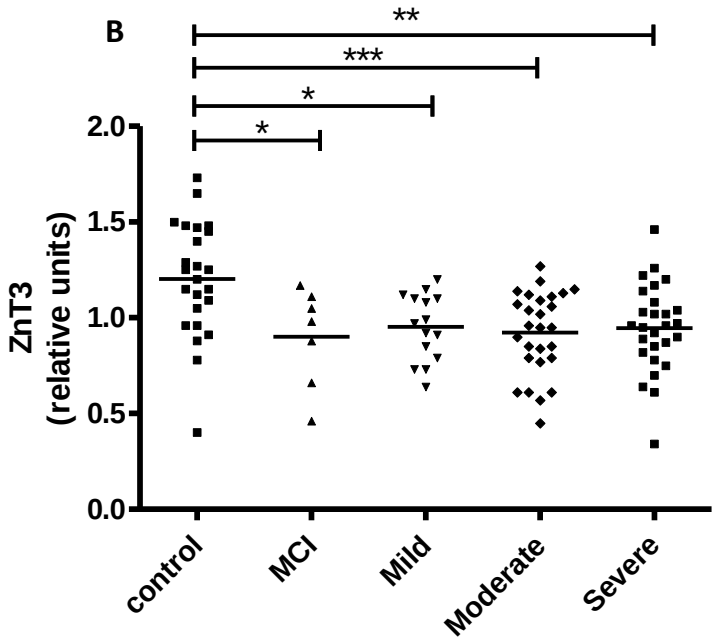
ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	51.236	4	12.809	6.994	.000[a]
	Residual	141.020	77	1.831		
	Total	192.256	81			

Dependent Variable: MMSE group

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.063	1.112		3.655	.000
	Btub (relative units)	-.914	1.202	-.080	-.760	.450
	ZnT3 (relative units)	-2.152	.594	-.377	-3.624	.001
	PSD95 (relative units)	-1.621	.685	-.246	-2.366	.021
	SPP (relative units)	.430	1.315	.035	.327	.744


B
**
***
*
*
2.0
1.5
1.0
0.5
0.0
ZnT3
(relative units)
control
MCI
Mild
Moderate
Severe
Classification of cognitive impairment

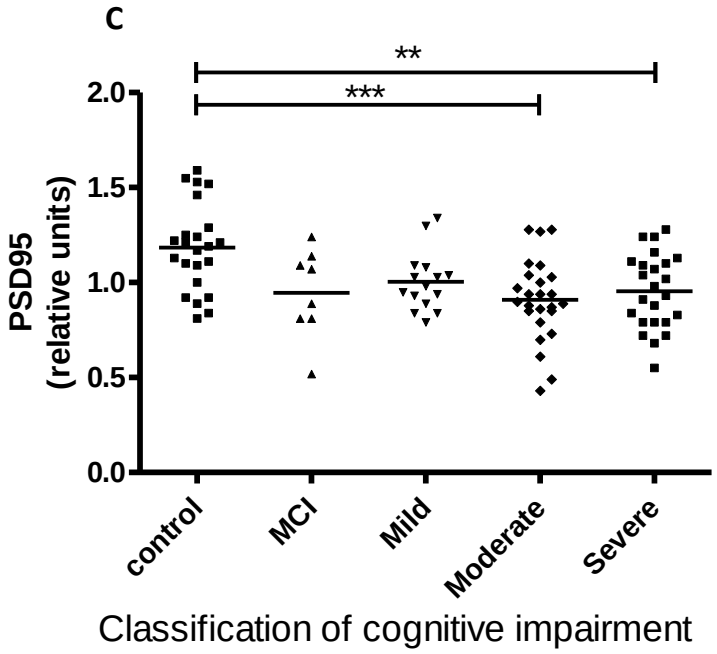

C
**
***
2.0
1.5
1.0
0.5
0.0
PSD95
(relative units)
control
MCI
Mild
Moderate
Severe
Classification of cognitive impairment

Figure 4.3.4 The ratio of ZnT3 to SPP in BA9, from semi-quantitative Western blotting, predicts the degree of cognitive impairment.

Regression analysis using the three protein ratio variables from BA9 (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) as independent predictor variables showed the ratio of ZnT3 to SPP to be a significant predictor of cognitive impairment (beta= -0.325, p=0.006). The ANOVA for the model was significant (p=0.003). The cognitive impairment groups were; 'controls' (assigned a value of 1 and including all designated control cases), 'MCI/normal' (dementia cases assigned a value of 2 and based on a MMSE score of 25 to 30), 'mild dementia' (assigned a value of 3 and based upon a MMSE score of 17 to 24), 'moderate dementia' (assigned a value of 4 and based upon a MMSE score of 10 to 16) and 'severe dementia' (assigned a value of 5 and based upon a MMSE score of 9 or less). The difference in the distribution of the three protein ratios between cognitive impairment groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed the ratio of ZnT3 to SPP to be significantly higher in control cases compared to cases with severe cognitive impairment (p=0.003), moderate cognitive impairment (p=0.009) and cases classified as MCI/normal (p=0.04). The ANOVA values were; F=4.886, df=4,88, p=0.001. The ratio of PSD95 to Btub was significantly higher in control cases compared to cases with moderate cognitive impairment (p=0.01). The ANOVA values were; F=3.388, df=4,87, p=0.013. The horizontal bars within the data points in the graph represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.406[a]	.165	.133	1.43468

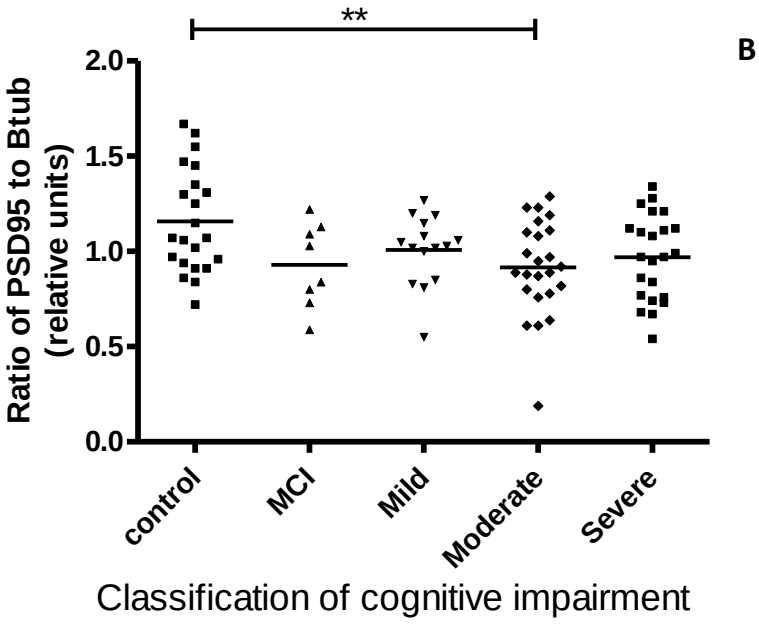
ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	31.708	3	10.569	5.135	.003[a]
	Residual	160.548	78	2.058		
	Total	192.256	81			

Dependent Variable: MMSE group

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	6.779	1.268		5.346	.000
	Ratio of SPP to Btub	-.933	1.036	-.106	-.900	.371
	Ratio of PSD94 to Btub	-1.064	.689	-.178	-1.544	.127
	Ratio of ZnT3 to SPP	-3.582	1.263	-.325	-2.837	.006


B
**
2.0
1.5
1.0
0.5
0.0
Ratio of PSD95 to Btub
(relative units)
control
MCI
Mild
Moderate
Severe
Classification of cognitive impairment

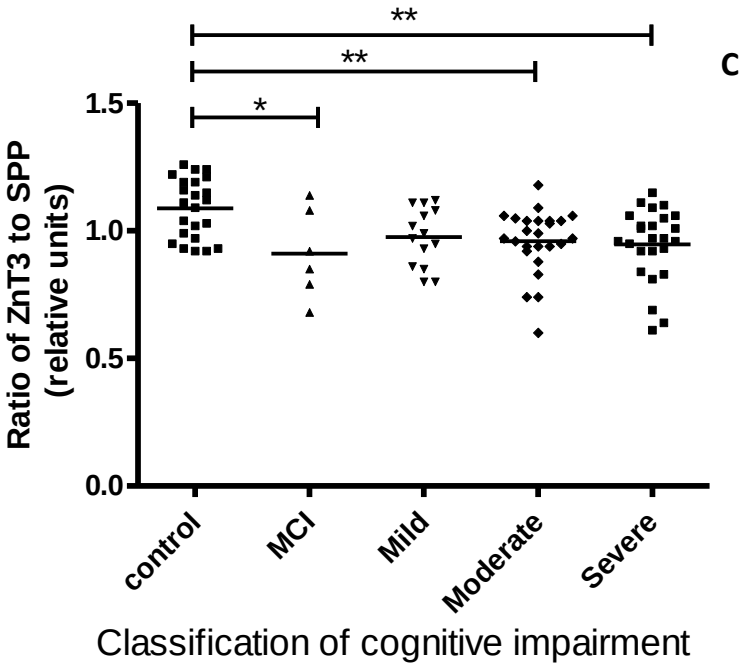

C
**
**
*
1.5
1.0
0.5
0.0
Ratio of ZnT3 to SPP
(relative units)
control
MCI
Mild
Moderate
Severe
Classification of cognitive impairment

Figure 4.3.5. ZnT3 values in BA40 predict disease severity, based upon the classification of cognitive impairment.

Regression analysis using the values for Btub, PSD95, SPP and ZnT3 from BA40 as independent predictor variables showed ZnT3 values to be a significant predictor of the cognitive impairment group (beta= -0.415, p=0.003), used as an indicator of disease severity. The ANOVA for the model was significant (p=0.004). The cognitive impairment groups were; 'controls' (assigned a value of 1 and including all designated control cases), 'MCI/normal' (dementia cases assigned a value of 2 and based on a MMSE score of 25 to 30), 'mild dementia' (assigned a value of 3 and based upon a MMSE score of 17 to 24), 'moderate dementia' (assigned a value of 4 and based upon a MMSE score of 10 to 16) and 'severe dementia' (assigned a value of 5 and based upon a MMSE score of 9 or less). The difference ZnT3 values between cognitive impairment groups was not found to be significantly different according to a Kruskal Wallis test (p=0.093). The scatter plot depicts the relationship between ZnT3 values in the cognitive impairment groups.

Model Summary

A

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.463[a]	.215	.164	1.42208

ANOVA[b]

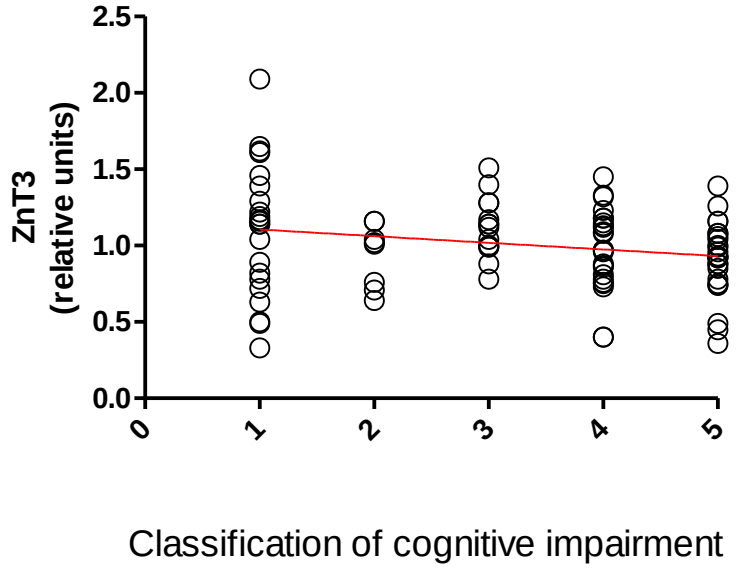
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	34.258	4	8.565	4.235	.004[a]
	Residual	125.384	62	2.022		
	Total	159.642	66			

Dependent Variable: MMSE group

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.964	.808		4.907	.000
	SPP (relative units)	-.716	.930	-.109	-.770	.444
	PSD95 (relative units)	.340	.984	.050	.346	.731
	Btub (relative units)	-.605	.666	-.121	-.907	.368
	ZnT3 (relative units)	-2.197	.700	-.415	-3.139	.003

B


ZnT3
(relative units)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
Classification of cognitive impairment

C

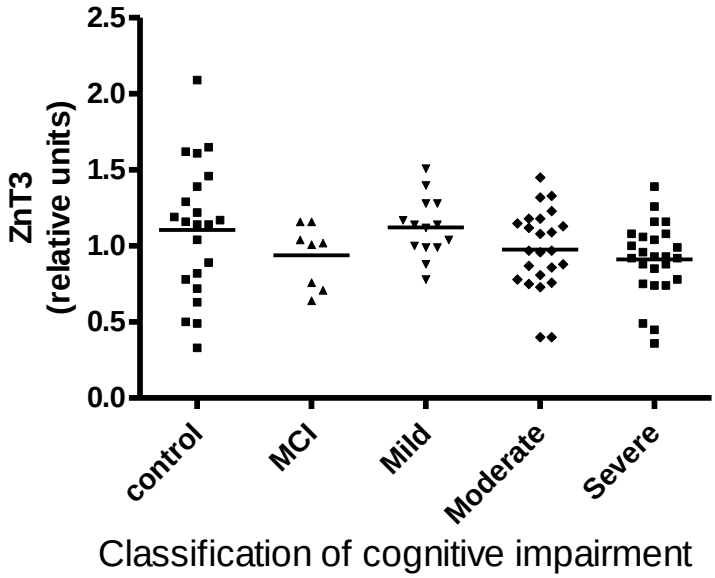

ZnT3
(relative units)
2.5
2.0
1.5
1.0
0.5
0.0
control
MCI
Mild
Moderate
Severe
Classification of cognitive impairment

Figure 4.3.6 The ratio of ZnT3 to SPP in BA40, from semi-quantitative Western blotting, predicts the degree of cognitive impairment.

Regression analysis using the three protein ratio variables from BA40 (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) as independent predictor variables showed the ratio of ZnT3 to SPP to be a significant predictor of cognitive impairment (beta= -0.311, p=0.021). The ANOVA for the model was significant (p=0.032). The cognitive impairment groups were; 'controls' (assigned a value of 1 and including all designated control cases), 'MCI/normal' (dementia cases assigned a value of 2 and based on a MMSE score of 25 to 30), 'mild dementia' (assigned a value of 3 and based upon a MMSE score of 17 to 24), 'moderate dementia' (assigned a value of 4 and based upon a MMSE score of 10 to 16) and 'severe dementia' (assigned a value of 5 and based upon a MMSE score of 9 or less). The difference in the distribution of the three protein ratios between cognitive impairment groups was not significantly different. The horizontal bars within the data points in the graph represent the mean values.

Model Summary

A

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.359[a]	.129	.088	1.48549

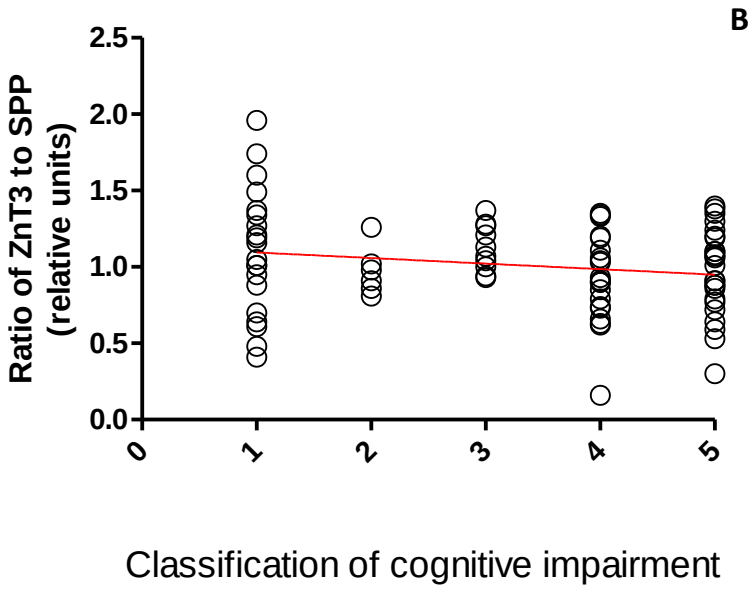
ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	20.620	3	6.873	3.115	.032[a]
	Residual	139.021	63	2.207		
	Total	159.642	66			

Dependent Variable: MMSE group

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.287	.184		17.890	.000
	Ratio of SPP to Btub	1.915	1.614	.169	1.187	.240
	Ratio of ZnT3 to SPP	-1.638	.693	-.311	-2.365	.021
	Ratio of PSD95 to Btub	-.041	.890	-.007	-.046	.964


B
Ratio of ZnT3 to SPP
(relative units)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
Classification of cognitive impairment

4.4 Relationships between pathology, and synaptic and neuronal biochemistry.

When investigating the relationships between the pathology data; in the form of semi-quantitative scores for Aβ staining (plaque pathology), tau staining (tangle pathology) and α-synuclein staining/pathology, and the biochemical data, the reciprocal nature of this relationship became apparent. Many of the synaptic proteins measured in this study were decreased in cases with more severe pathology scores, yet there are biological arguments for neuronal proteins precipitating aggregation of pathological proteins, as there are counter arguments for pathological deposits causing damage to, and reductions in, synaptic and neuronal proteins. Thus, there is a valid case for using pathology scores as predictors of synaptic biochemistry values and *vice versa*. However, in the majority of instances only one combination gave a statistically significant result, and this has been reported. The exception was the relationship between tangles and PSD95 and Btub in BA40, in which the prediction was reciprocal. Therefore the stronger relationship, both in terms of statistics and biological rationale, was reported.

Regression analysis was performed using the semi-quantitative scores for the three pathology types (plaques, tangles and α-synuclein) in BA9 as independent predictor variables for each of the proteins of interest (Btub, PSD95, SPP and ZnT3) derived from semi-quantitative Western blotting. This established the tangle score to be a significant predictor of SPP values in BA9 – with a direct relationship, indicated by the positive beta value, such that a decrease in the tangle score related to a decrease in the SPP value. Figure 4.4.1 shows the values from the regression analysis and a scatter plot depicting SPP values according to tangle score group, whilst a trend of increasing SPP values with increasing tangle score can be detected it was not statistically significant according to post hoc test.

Regression analysis using the values for the proteins of interest as predictors of pathology did not reveal any significant relationships. However, similar analysis using the ratios of SPP to Btub, PSD95

to Btub and ZnT3 to SPP as independent predictor variables for each of the types of semi-quantitative pathology scores found the ratio of ZnT3 to SPP in BA9 to predict both the tangle and plaque scores. Figure 4.4.2 shows the inverse relationship between the ratio of ZnT3 to SPP and the plaque score in BA9 – evinced by the negative beta value from the regression analysis, and depicted graphically by the scatter plot showing a significantly lower ratio of ZnT3 to SPP in cases with severe tangle score to cases with a plaque score of moderate or none. Similarly, figure 4.4.3 shows the inverse relationship between the ratio of ZnT3 to SPP and tangle score, with a table of output from the regression analysis and a scatter plot illustrating the significantly higher ratio of ZnT3 to SPP in cases with no tangles compared to cases with moderate of severe tangle scores. None of the pathologies significantly predicted any of the protein ratios.

Figure 4.4.1. The semi-quantitative tangle score predicted SPP values (from semi-quantitative Western blotting) in BA9.

Regression analysis, using the semi-quantitative scores for the three types of pathology assessed in each brain region (Aβ, tau and α-synuclein) as independent predictor variables, showed the tangle (tau) score to be a significant predictor of SPP values (obtained from semi-quantitative Western blotting) in BA9 (beta= 0.319, p=0.011). The ANOVA for the regression model was significant (p=0.016). The difference in SPP values between tangle score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, whilst the ANOVA was significant – the post hoc test found no differences between specific groups (F=2.956, df=3,99, p=0.036). The horizontal bars within the data points in the graph represent the mean values.

Model Summary

A

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.319[a]	.101	.073	.12654

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.174	3	.058	3.614	.016[a]
	Residual	1.537	96	.016		
	Total	1.711	99			

b. Dependent Variable: SPP (relative units)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.051	.022		-2.300	.024
	plaque score for BA9	-.001	.013	-.008	-.069	.945
	tangle score for BA9	.041	.016	.319	2.603	.011
	alpha synuclein score for BA9	-.011	.014	-.080	-.825	.412

a. Dependent Variable: SPP (relative units)

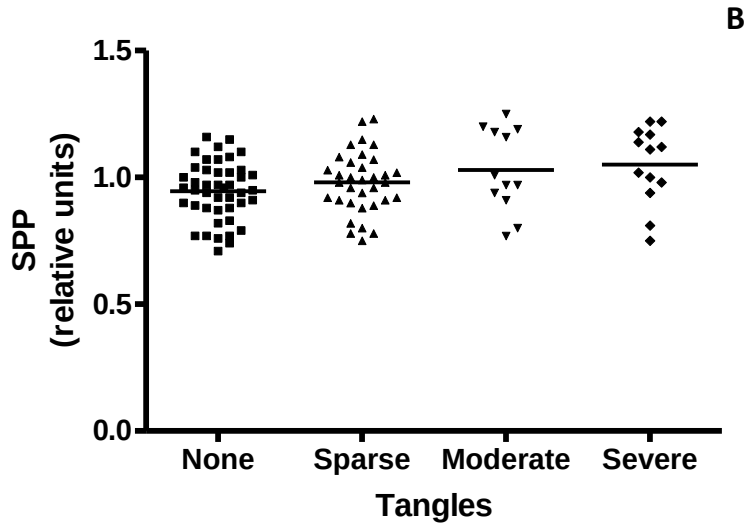

B
1.5
1.0
0.5
0.0
SPP
(relative units)
None
Sparse
Moderate
Severe
Tangles

Figure 4.4.2 The ratio of ZnT3 to SPP (relative units), from semi-quantitative Western blotting, predicted the plaque score in BA9

Regression analysis using the 3 protein ratio variables from BA9 (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) as independent predictor variables showed the ratio of ZnT3 to SPP to be a significant predictor of the semi-quantitative plaque score in BA9 (beta= -0.323, p=0.006). The ANOVA for the model was significant (p=0.002). The difference between plaque score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed the ratio of ZnT3 to SPP to be significantly lower in cases with a severe plaque score compared to cases with a plaque score of absent and moderate (p=0.001, p=0.027), for the ANOVA; F=5.893, (df=3,94), p=0.001. The horizontal bars within the data points in the graph represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.403[a]	.163	.132	1.125

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	19.900	3	6.633	5.242	.002[a]
	Residual	102.500	81	1.265		
	Total	122.400	84			

b. Dependent Variable: plaque score for BA9

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.449	.123		11.807	.000
	Ratio of SPP to Btub (relative units)	-.068	.786	-.010	-.087	.931
	Ratio of PSD to BTUB (relative units)	-.857	.537	-.183	-1.596	.114
	Ratio of ZnT3 to SPP (relative units)	-1.459	.513	-.323	-2.846	.006

a. Dependent Variable: plaque score for BA9

B

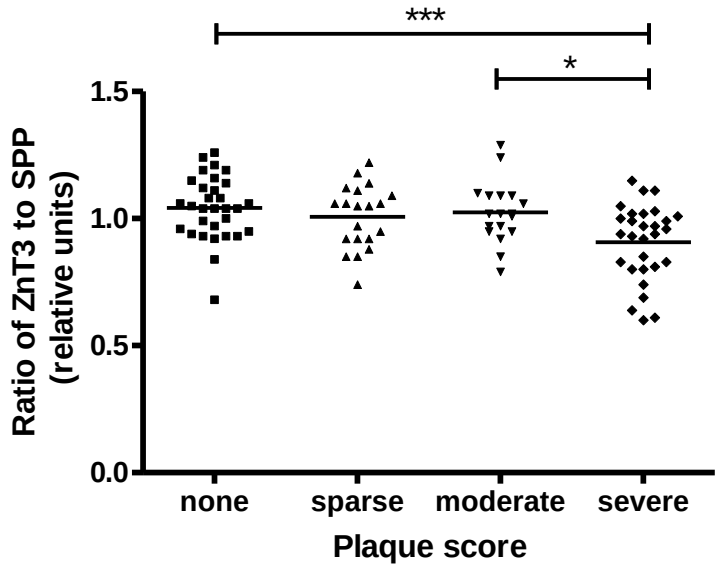

***
*
1.5
1.0
0.5
0.0
Ratio of ZnT3 to SPP
(relative units)
none
sparse
moderate
severe
Plaque score

Figure 4.4.3 The ratio of ZnT3 to SPP (relative units), from semi-quantitative Western blotting, predicted the tangle score in BA9

Regression analysis using the three protein ratio variables from BA9 (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) as independent predictor variables showed the ratio of ZnT3 to SPP to be a significant predictor of the semi-quantitative tangle score in BA9 (beta= -0.319, p=0.008). The ANOVA for the model was significant (p=0.023). The difference between plaque score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed the ratio of ZnT3 to SPP to be significantly lower in cases with a severe plaque score compared to cases with a plaque score of absent and moderate (p=0.001, p=0.027), for the ANOVA; F=5.893, (df=3,94), p=0.001. Analysis of the differences in ZnT3 to SPP ratio between tangle score groups was carried out using the non-parametric Mann Whitney U test as the homogeneity of variance was significant. Cases with no tangles had a significantly higher ratio of ZnT3 to SPP than cases with moderate or severe tangle scores (W=223, Z= -2.303, p=0.021 and W=253, Z= -2.28, p=0.023). The horizontal bars within the data points in the graph represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.330[a]	.109	.076	1.004

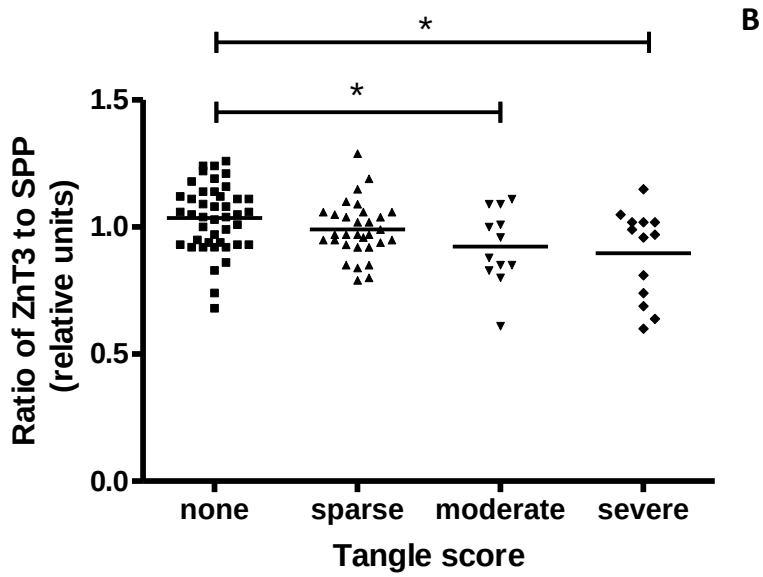
ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	10.101	3	3.367	3.337	.023[a]
	Residual	82.737	82	1.009		
	Total	92.837	85			

b. Dependent Variable: tangle score for BA9

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.904	.109		8.305	.000
	Ratio of SPP to Btub (relative units)	.189	.693	.033	.273	.786
	Ratio of PSD to BTUB (relative units)	.010	.482	.002	.020	.984
	Ratio of ZnT3 to SPP (relative units)	-1.248	.457	-.319	-2.728	.008


B
*
*
1.5
1.0
0.5
0.0
Ratio of ZnT3 to SPP
(relative units)
none
sparse
moderate
severe
Tangle score

The relationships between the pathology scores and proteins and protein ratios in BA24 were investigated in the same manner as in BA9, with the same combinations of variables (likewise for BA40). It was found that the values for PSD95 and ZnT3 significantly and independently predicted both the plaque score and the tangle score in BA24. Figure 4.4.4 shows the regression output for prediction of plaque score. PSD95 had a direct relationship to plaque score whilst ZnT3 had an inverse relationship; this was also the case for tangle scores – shown in figure 4.4.5. The scatter plots in figure 4.4.4 reflect this; showing that there was no significant difference between PSD95 values for the different plaque scores – yet a trend of increasing PSD95 value as plaque score increased. Conversely, the scatter plot for ZnT3 shows significantly lower ZnT3 values in cases with a plaque score of severe compared to cases with a plaque score of none.

The relationship between PSD95 and ZnT3 values and tangle score is shown in figure 4.4.5. The scatter plots illustrate the non-significant trends present for both proteins which mirror the positive and negative beta values for PSD95 and ZnT3 respectively – with PSD95 values increasing as tangle score increases – and vice versa for ZnT3 values.

Figure 4.4.4 PSD95 and ZnT3 levels, from semi-quantitative Western blotting, predict plaque scores in BA24.

Regression analysis, using the values obtained from semi-quantitative Western blotting for the proteins of interest (Btub, PSD95, SPP and ZnT3) as independent predictor variables, showed PSD95 and ZnT3 to be significant predictors of the semi-quantitative plaque score in BA24 (beta= 0.251, p=0.034 and beta= -0.346, p=0.003). The ANOVA for the model was significant (p=0.01). The difference in ZnT3 and PSD95 values between plaque score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed ZnT3 values to be significantly lower in cases with a severe plaque score compared to cases with a plaque score of absent (p=0.029), for the ANOVA; F=3.331, (df=3,91), p=0.023. There was no significant difference in PSD95 values between plaque score groups. The horizontal bars within the data points in the graphs represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.398[a]	.158	.114	.997

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	14.201	4	3.550	3.569	.010[a]
	Residual	75.602	76	.995		
	Total	89.802	80			

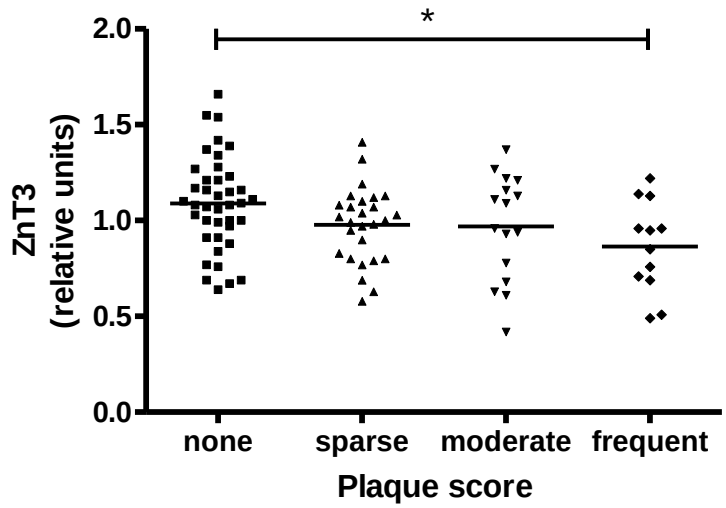
b. Dependent Variable: plaque score for BA24

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.259	.393		.657	.513
	PSD95 (relative units)	1.110	.513	.251	2.163	.034
	ZnT3 (relative units)	-1.514	.484	-.346	-3.127	.003
	Btub (relative units)	.809	.855	.108	.947	.347
	SPP (relative units)	-.023	.118	-.022	-.196	.845

a. Dependent Variable: plaque score for BA24

B


*
2.0
1.5
1.0
0.5
0.0
ZnT3
(relative units)
none
sparse
moderate
frequent
Plaque score

C

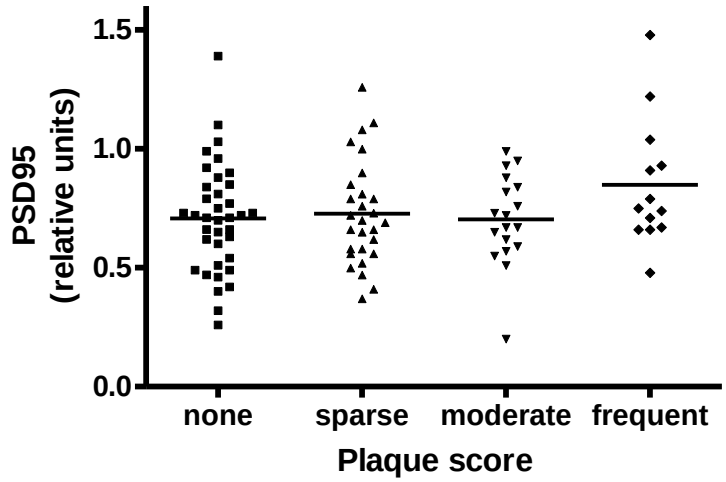

1.5
1.0
0.5
0.0
PSD95
(relative units)
none
sparse
moderate
frequent
Plaque score

Figure 4.4.5 PSD95 and ZnT3 values, from semi-quantitative Western blotting, predicted tangle score in BA24.

Regression analysis, using the values obtained from semi-quantitative Western blotting for the proteins of interest (Btub, PSD95, SPP and ZnT3) as independent predictor variables, showed PSD95 and ZnT3 to be significant predictors of the semi-quantitative tangle score in BA24 (beta= 0.337, p=0.003 and beta= -0.303, p=0.006). The ANOVA for the model was significant (p=0.007). The difference in ZnT3 and PSD95 values between tangle score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed no significant difference in the values of either protein between tangle score groups. The horizontal bars within the data points in the graphs represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.403[a]	.162	.120	.876

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.750	4	2.938	3.831	.007[a]
	Residual	60.571	79	.767		
	Total	72.321	83			

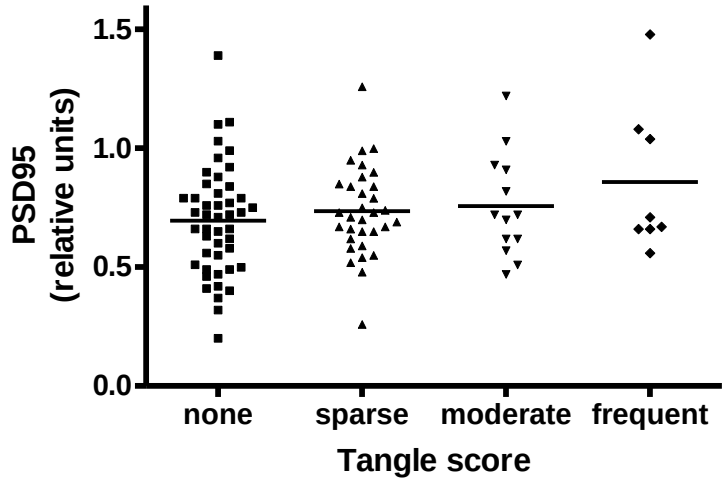
b. Dependent Variable: tangle score for BA24

Coefficients[a]

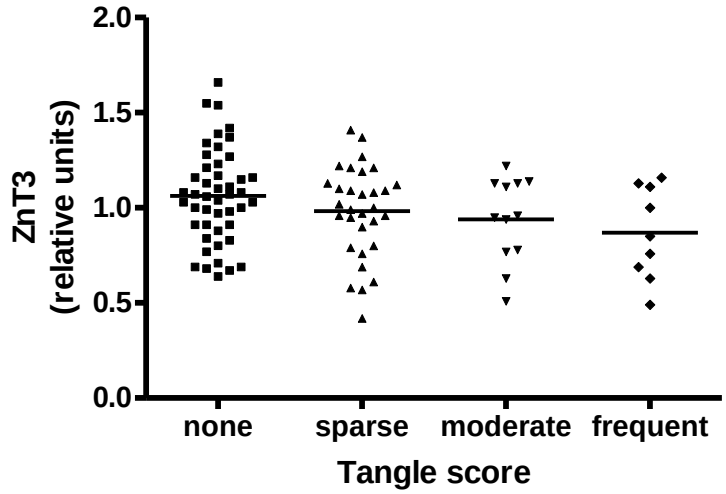
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-.143	.337		-.423	.673
	PSD95 (relative units)	1.332	.442	.337	3.016	.003
	ZnT3 (relative units)	-1.166	.415	-.303	-2.810	.006
	Btub (relative units)	.356	.742	.054	.480	.632
	SPP (relative units)	-.032	.101	-.035	-.317	.752

a. Dependent Variable: tangle score for BA24

B


PSD95
(relative units)
1.5
1.0
0.5
0.0
none
sparse
moderate
frequent
Tangle score

C


ZnT3
(relative units)
2.0
1.5
1.0
0.5
0.0
none
sparse
moderate
frequent
Tangle score

Btub and tangles have an intertwined relationship in BA40 – with the values (or score) for each predicting the other. Figure 4.4.6 contains the output from the regression analysis in which Btub values significantly predicted tangle score in BA40 in an inverse manner, and a scatter plot depicting the significantly lower Btub values found in cases with a tangle score of severe compared to cases with a tangle score of none or sparse. Figure 4.4.7 illustrates the reciprocal relationship in which the tangle score in BA40 significantly predicted Btub values. In addition, it was found that the tangle score in BA40 significantly predicted PSD95 values – this is combined in the same figure.

Figure 4.4.6 The semi-quantitative tangle score predicted PSD95 and Btub values (obtained from semi-quantitative Western blotting) in BA40.

Regression analysis, using the semi-quantitative scores for the three types of pathology assessed in each brain region (Aβ, tau and α-synuclein) as independent predictor variables, showed the tangle (tau) score to be a significant predictor of PSD95 and Btub values (obtained from semi-quantitative Western blotting) in BA40 (beta= -0.400, p=0.003 and beta= -418, p=0.002). The ANOVAs for both regression models were significant (p=0.001). The difference in PSD95 values between tangle score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which showed cases with no tangles to have higher PSD95 levels than cases with a tangle score of frequent or moderate (p=0.003 and p=0.006); for the ANOVA, F=7.456, (df=3,83), p<0.001. The differences between Btub values for tangle scores categories is described statistically and depicted graphically in the preceding figure. The horizontal bars within the data points in the graph represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.432[a]	.186	.155	.19810757

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.701	3	.234	5.955	.001[b]
	Residual	3.061	78	.039		
	Total	3.762	81			

a. Dependent Variable: PSD95 (relative units)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	.072	.035		2.055	.043
	plaque score for BA40	-.009	.024	-.051	-.394	.695
	tangle score for BA40	-.082	.027	-.400	-3.105	.003
	alpha synuclein score for BA40	.006	.030	.022	.209	.835

B

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.403[a]	.162	.134	.26356880

ANOVA[a]

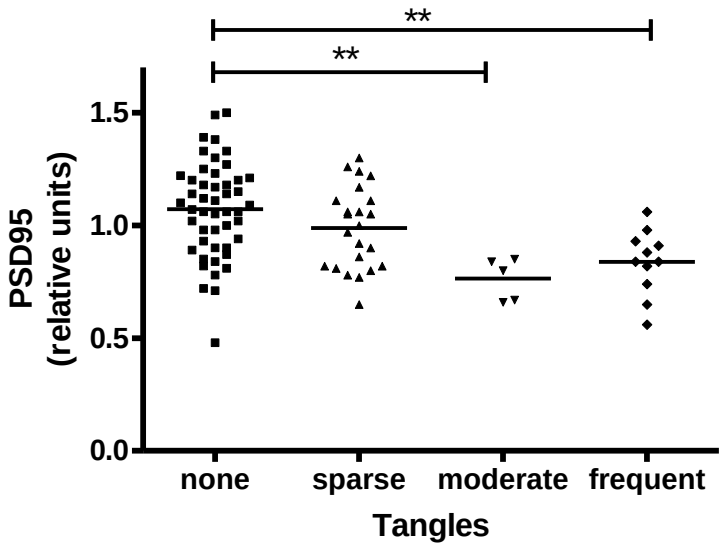
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.186	3	.395	5.690	.001[b]
	Residual	6.113	88	.069		
	Total	7.299	91			

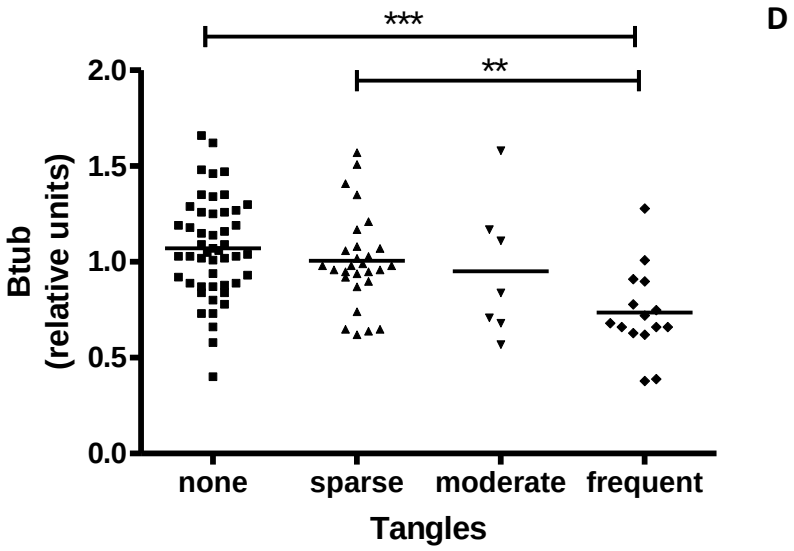
a. Dependent Variable: Btub (relative units)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.047	.045		1.061	.292
	plaque score for BA40	.009	.032	.039	.299	.766
	tangle score for BA40	-.108	.034	-.418	-3.220	.002
	alpha synuclein score for BA40	.043	.036	.119	1.198	.234

C





To determine the relationship between the protein ratios and pathology in BA40 regression analysis was performed using the protein ratios as predictor variables for the pathology scores and vice versa. It was found that the tangle score was significantly predicted by both the ratio of SPP to Btub (in a direct manner) and of PSD95 to Btub (in an inverse manner) – the values for the regression analysis are shown in figure 4.4.8, as are scatter plots depicting the distribution of the ratios of SPP to Btub and PSD95 to Btub according to tangle score.

It was also found that the ratio of ZnT3 to SPP significantly predicted the α-synuclein score in BA40 in an inverse manner - the values for this regression analysis are shown in figure 4.4.9, as is a scatter plot depicting the distribution of the ratio of ZnT2 to SPP according to α-synuclein score.

Figure 4.4.7 The ratio of SPP to Btub and PSD95 to Btub, obtained from semi-quantitative Western blot analysis, predicted the tangle score in BA40

Regression analysis using the three protein ratio variables from BA40 (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) as independent predictor variables showed the ratio of SPP to Btub, and the ratio of PSD95 to Btub, to be significant predictors of the semi-quantitative tangle score in BA40 (beta= 0.467, p=0.002 and beta= -0.361, p=0.023). The ANOVA for the model was significant (p=0.011). The differences in the ratio of SPP to Btub, and PSD95 to Btub, between tangle score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed no significant differences. The horizontal bars within the data points in the graphs represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.393[a]	.155	.116	.981

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	11.621	3	3.874	4.021	.011[b]
	Residual	63.579	66	.963		
	Total	75.200	69			

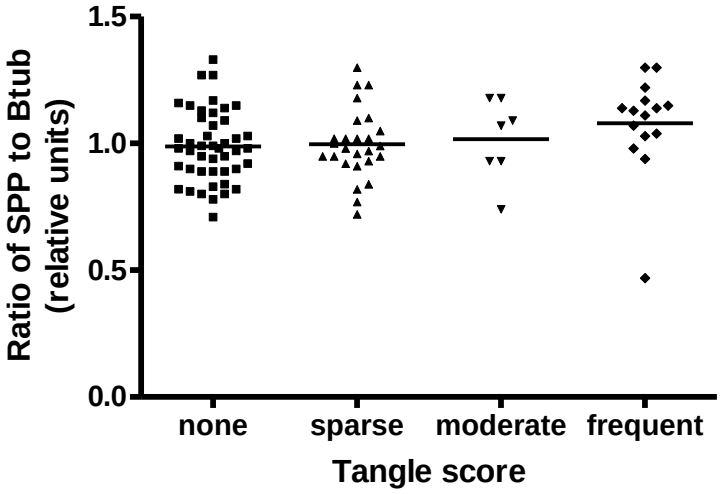
a. Dependent Variable: tangle score for BA40

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.789	.118		6.671	.000
	Ratio of SPP to Btub (relative units)	3.511	1.065	.467	3.298	.002
	Ratio of ZnT3 to SPP (relative units)	.123	.453	.035	.271	.787
	Ratio of PSD95 to Btub (relative units)	-1.391	.596	-.361	-2.335	.023

a. Dependent Variable: tangle score for BA40

B


Ratio of SPP to Btub
(relative units)
1.5
1.0
0.5
0.0
none
sparse
moderate
frequent
Tangle score

C

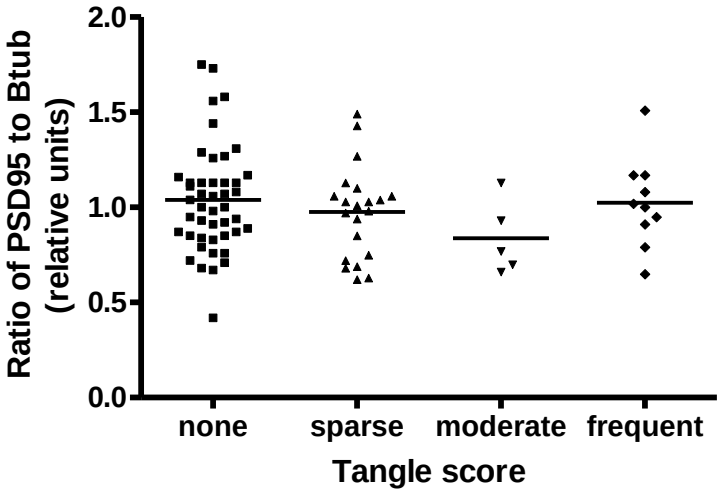

Ratio of PSD95 to Btub
(relative units)
2.0
1.5
1.0
0.5
0.0
none
sparse
moderate
frequent
Tangle score

Figure 4.4.8 The ratio of ZnT3 to SPP, obtained from semi-quantitative Western blotting, predicted the α-synuclein score for BA40.

Regression analysis using the 3 protein ratio variables from BA40 (SPP to Btub, PSD95 to Btub and ZnT3 to SPP) as independent predictor variables showed the ratio of ZnT3 to SPP to be a significant predictor of the semi-quantitative alpha synuclein score in BA40 (beta= -0.430, p=0.002). The ANOVA for the model was significant (p=0.008). The differences in the ratio of ZnT3 to SPP between alpha synuclein score groups was analysed by one-way ANOVA and the Bonferroni post-hoc test, which revealed no significant differences. The horizontal bars within the data points in the graph represent the mean values.

A

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.410[a]	.168	.129	.691

ANOVA[a]

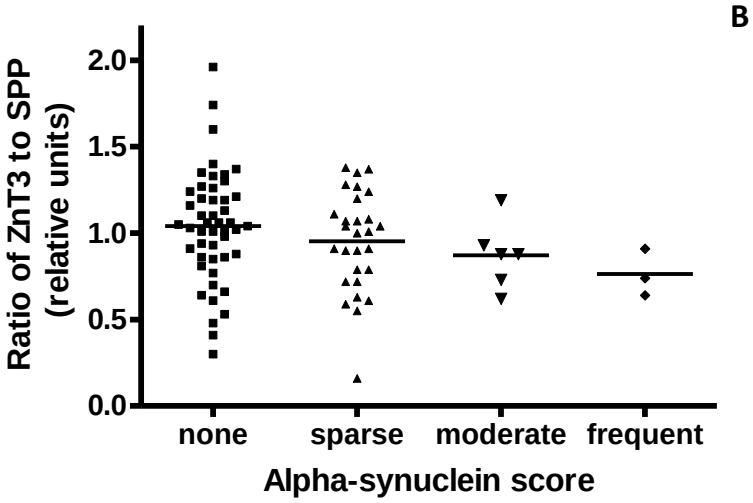
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	6.172	3	2.057	4.304	.008[b]
	Residual	30.592	64	.478		
	Total	36.765	67			

a. Dependent Variable: alpha synuclein score for BA40

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.574	.084		6.812	.000
	Ratio of SPP to Btub (relative units)	.331	.764	.062	.434	.666
	Ratio of ZnT3 to SPP (relative units)	-1.084	.327	-.430	-3.313	.002
	Ratio of PSD95 to Btub (relative units)	.304	.425	.112	.715	.477

a. Dependent Variable: alpha synuclein score for BA40


B
Ratio of ZnT3 to SPP
(relative units)
2.0
1.5
1.0
0.5
0.0
none
sparse
moderate
frequent
Alpha-synuclein score

4.5 Measurement of drebrin in BA9 by ELISA and its relationship to alpha synuclein pathology.

The relationship between drebrin concentration, quantified by ELISA in BA9, and pathology was determined by regression analysis. It was found that the semi-quantitative α-synuclein score in BA9 significantly predicted drebrin concentration (expressed as the log10 of the ng per µg of total protein) in a direct manner – evinced by the positive beta coefficient shown in the table of regression values in figure 4.5.1. The scatter plot in figure 4.5.1 shows the trend of drebrin concentration increasing with α-synculein score.

Drebrin concentration was not significantly different between control, DLB, PDD and AD cases despite higher mean values for DLB and PDD than control and AD. However, when PDD and DLB cases were combined into a Lewy body dementia (LBD) group, the drebrin concentration was significantly higher in LBD cases than AD cases – shown in figure 4.5.2.

Figure 4.5.1. In BA9 the semi-quantitative α-synuclein score significantly predicted drebrin values obtained from ELISA.

The relationship between the three pathologies (α-synuclein, plaques and tangles) and drebrin concentration in BA9 was analysed by multiple regression (image A). The ANOVA for the model was significant (p=0.002). The α-synuclein score significantly predicted drebrin concentration (beta=0.449, p<0.001). The scatter plot (image B) shows the distribution of drebrin values according to α-synuclein score, with a linear regression line. Note that there were only two cases with drebrin values in the 'severe α-synuclein pathology' group and so these were combined with the moderate α-synuclein pathology group.

Model Summary

A

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.455[a]	.207	.170	44.57498

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	33266.602	3	11088.867	5.581	.002[a]
	Residual	127163.424	64	1986.929		
	Total	160430.026	67			

Dependent Variable: Drebrin BA9 ng/ug total protein

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	90.697	9.449		9.599	.000
	plaque score for BA9	.825	5.986	.020	.138	.891
	tangle score for BA9	-2.898	6.605	-.064	-.439	.662
	alpha synuclein score for BA9	29.649	7.415	.449	3.998	.000

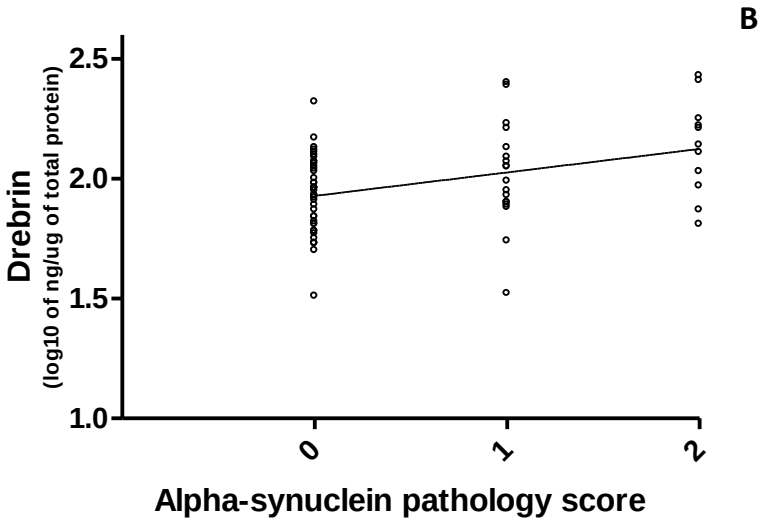
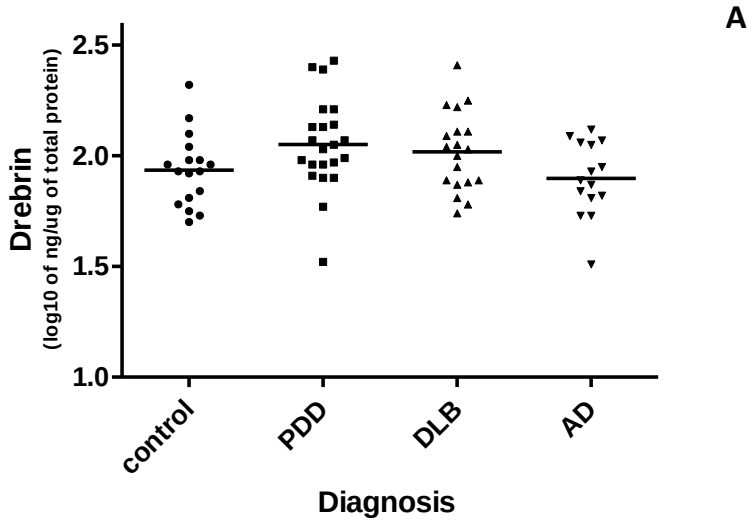
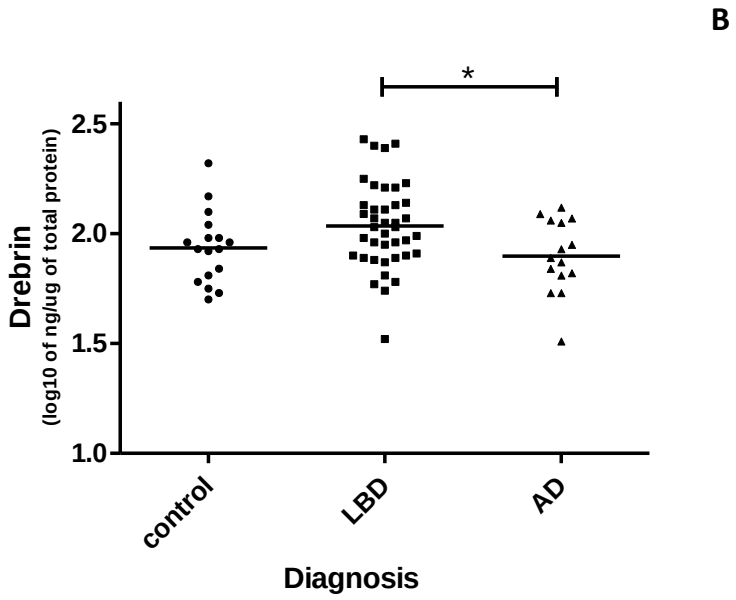

B
Drebrin
(log10 of ng/ug of total protein)
2.5
2.0
1.5
1.0
0
1
2
Alpha-synuclein pathology score

Figure 4.5.2. Drebrin concentration in BA9 according to clinical diagnosis.

The difference in drebrin concentration (measured by ELISA and expressed as the log10 of the ng per µg of total protein per case) between diagnostic groups was analysed by one-way ANOVA and Bonferroni post-hoc test. There was no significant difference between standard diagnostic groups (shown in the top graph) – despite a trend of higher concentrations in PDD and DLB cases compared to control and AD cases. However, when values for PDD and DLB cases were combined into a Lewy body dementia (LBD) group, drebrin concentration was significantly higher in cases in this group compared to AD cases (see bottom graph) p=0.045 (ANOVA values; F=3.901, df=2,70, p=0.025). The horizontal bars within data points represent the mean values.





4.6 Immunohistochemical investigation of the expression of ZnT3

Immunohistochemistry was used to investigate the expression of ZnT3 in paraffin embedded sections of post-mortem tissue from various cortical regions (see figures 3.6.1 and 3.6.2 for details) from individuals with one of the three dementias of interest and healthy controls. Whilst the Znt3 antibody was undergoing optimisation to confirm the presence of specific staining and establish a protocol an unexpected pattern of staining was observed. This is illustrated in figure 4.6.1, where it can be seen that in the cortex of the Lewy body disease there was a perinuclear pattern of ZnT3 staining, something not seen in the control cases. ZnT3 expression has not been reported in human cortex, but an image from the only other study reporting a perinuclear expression of ZnT3 (in the MOCHA mouse, a model for lysosomal storage disorders) has been included for the purposes of comparison (Stoltenberg et al., 2004).

Further staining was performed for ZnT3 in sections taken from BA9 from the four diagnostic groups (PDD, DLB, AD and control) with the intention of further investigating the perinuclear staining and of corroborating the Western blotting data showing decreased ZnT3 levels in BA9. Unfortunately, the perinuclear pattern of ZnT3 staining was not duplicated in other cases and brain regions but a reduction in immuno-labelling of ZnT3 was observed in PDD BA9 (see image B, figure 4.6.2).

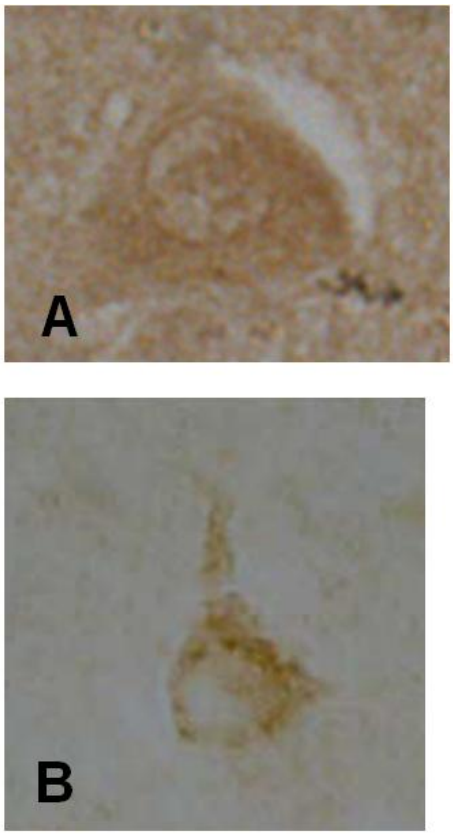


Figure 4.6.1. Altered ZnT3 expression in LBD cortex.

ZnT3 expression was investigated using DAB immuno-staining. Image A shows perinuclear expression of ZnT3 in the temporal cortex of a Lewy Body disease cases. Image B (adapted from Stoltenberg et al.) shows abnormal perinuclear ZnT3 expression from a mice model of a lysosomal storage pool deficiency syndrome (MOCHA mice) (Stoltenberg et al., 2004). The section featured in image A was counter-stained with haematoxylin.

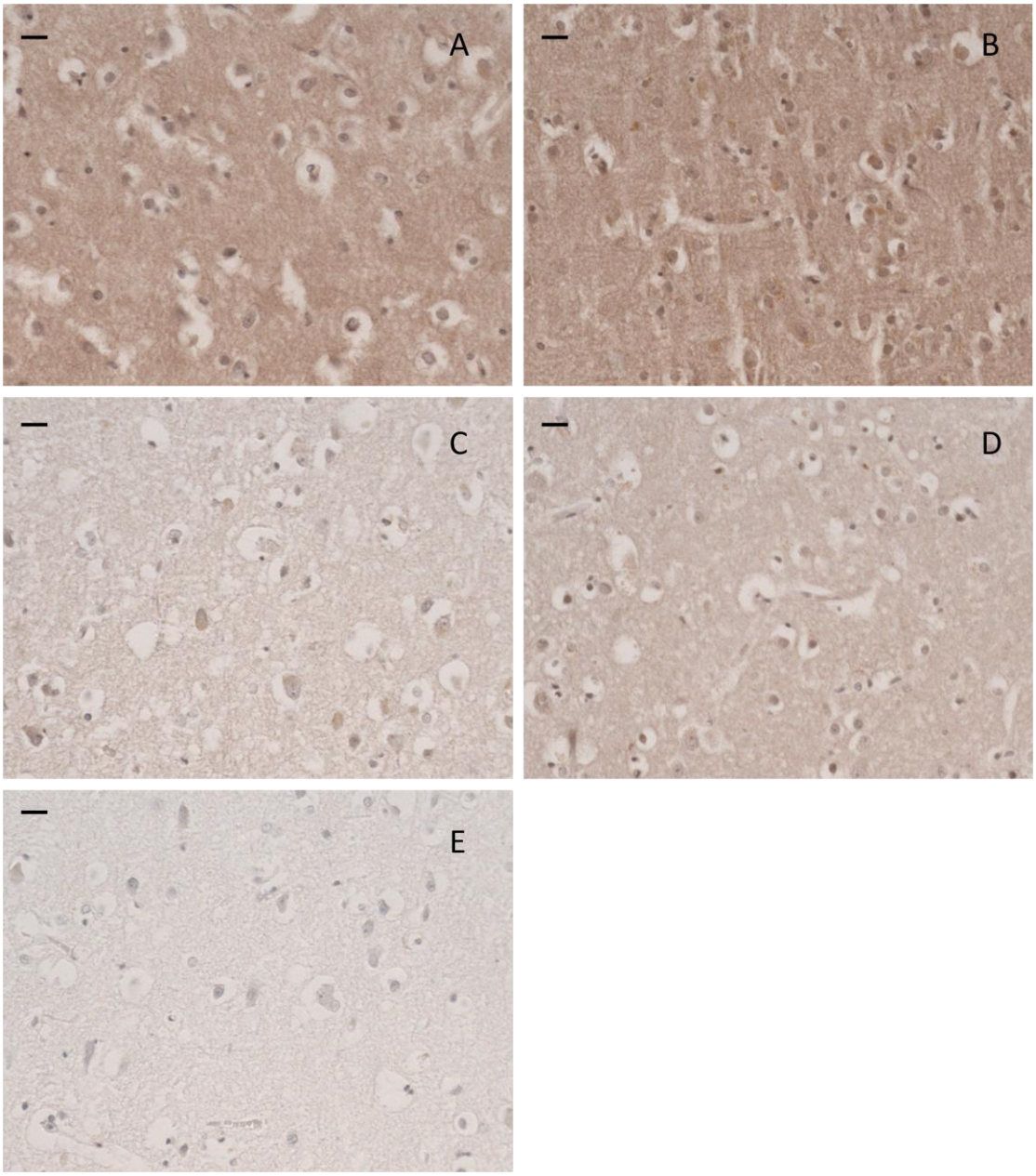


Figure 4.6.2: Examples of ZnT3 immuno-labelling in prefrontal cortex according to diagnosis

Coronal sections of BA9 were labelled for ZnT3 (images A-E, A=control, B=AD, C=PDD, D=DLB, E=no primary antibody). It can be seen that PDD sections had very low levels of neuropil staining, almost comparable to no primary antibody, and that DLB cases had slightly lower staining than control and AD. Scale bar = 10 µm and all images are 40X.

5 Discussion

In order to test the hypotheses, first the behavioural, cognitive and pathological characteristics of the cohort were examined to determine what underlying trends and differences between diagnostic groups were present. Furthermore, as has been stated in the hypothesis; it is not yet clear whether pathology is an independent predictor of clinical symptoms or whether synaptic dysfunction is driving synaptic pathology or *vice versa*, thus the predictive relationship between pathology and cognitive and behavioural symptoms was investigated.

5.1 The clinical and pathological characteristics of the cohort, according to diagnostic group.

Agitation and depression predicted cognitive impairment in a negative manner, and both were significantly lower in cases without cognitive impairment. As cases in the 'moderately impaired cognition' group can be interpreted as individuals who died at an earlier stage during the progression of cognitive impairment, this latter point provides evidence that depression and agitation are prevalent at an intermediate stage of dementia when cognition is not severely impaired and, whilst the difference between these scores in moderate and severely impaired cognition is not significant – the regression analysis supports the idea that depression and agitation worsen as does cognitive impairment.

That depression and agitation scores are so similar in their relationship to cognition can be explained to a large extent by their inter-correlation, something which is to be expected given the frequent co-morbidity of these symptoms. Indeed it has been suggested that agitation is a manifestation of depression in individuals with cognitive impairment of a magnitude that prevents expression of their depression by conventional means (Hall and O'Connor, 2004).

5.1.1 Pathological characteristics of the study cohort

Several key observations can be made regarding the pathology scores across the brain regions and diagnostic. Firstly, control cases had low levels of pathology, as would be expected, with the exception of Aβ pathology, for which some cases were categorised as severe. Upon a cursory examination, this may appear to contradict selection of these cases as controls. However, as detailed under the Aβ section of the introduction, Aβ pathology is frequently found in substantial quantities in otherwise normal and non-demented controls (Dickson et al., 1992; Jellinger and Attems, 2012; Knopman et al., 2003). One of the cornerstones of criticisms of the amyloid cascade hypothesis is the theory that Aβ is a damage response protein (Hardy, 2009). This is supported by its accumulation at the site of haemorrhages (Atwood et al., 2002; Cullen et al., 2006), and in the brains of boxers (dementia puglistica) and in the brains of individuals (generally soldiers) who have suffered blast injuries (Blennow et al., 2012).

Secondly, PDD cases had surprisingly little α-synuclein and tau pathology. Previous reports have found PDD cases to have a similar degree of cortical α-synuclein pathology to DLB cases (Harding and Halliday, 2001). Harding and Halliday examined LB pathology in the same cortical regions as this study but the number of cases for each diagnostic group was considerably lower. Indeed, cortical tau pathology has been reported in PDD with great regularity (Jellinger, 2011; Jellinger and Attems, 2008). Furthermore, tau and A-syn have been implicated on a molecular level by a study showing α-syn to induce phosphorylation of tau (Jensen et al., 1999).

However, the low cortical tau pathology observed in the PDD cohort is consistent with a report by Wills and colleagues, in which tau pathology was only encountered in the striatum of PDD cases, and not the inferior frontal gyrus (IFG) (Wills et al., 2010). The authors attribute this to difference to the higher oxidative stress, inherent in the striatal dopaminergic neurons, causing increased concentrations of phosphorylated GSK-3β (a kinase responsible for pathological phosphorylation of tau); an occurrence spared to the IFG, due to the lack of dopaminergic neurons and consequently lower oxidative stress.

Thirdly, DLB cases were characterised by universally high scores of all three types of pathology. This finding is in concordance with most reports of DLB pathology (McKeith et al., 2005), and in particular, with a recent comprehensive comparison of AD pathology in DLB and PDD which found DLB cases to have significantly more AD pathology than PDD (Jellinger and Attems, 2008). Indeed, tau pathology has been reported to provide a better correlate of dementia/cognitive impairment than α-syn pathology (Oinas et al., 2009).

Finally, AD cases were likewise characterised by high scores for Aβ and tau and relatively absent α-synuclein. The latter point is probably due to the selection criteria stipulated by this project for the AD cases, which required AD cases with little α-synuclein pathology in order that they might better serve to distinguish α-syn dementias from AD. Thus, it is not surprising that the AD cases in this study have α-syn pathology levels at variance with reports of an association between, and high prevalence of, α-syn and tau in AD (Arai et al., 2001; Hamilton, 2000). Indeed, it has been suggested that α-syn can seed tau precipitation through promotion of tau phosphorylation (Duka et al., 2006).

The validity of the semi-quantitative immunohistochemical staining and scoring of these pathologies is sound due to the multi-centre nature, inter-scorer reliability and simplicity of the scoring system. Indeed, semi-quantitative scales have been shown to be superior to more formal pathology scoring systems due to the ease of comparison between pathology types and different studies (Compta et al., 2011). Ultimately, the discrepancies revealed between the PDD pathology in this study and that of previous investigations serve to highlight the inherent variance of human studies and the need for ever larger cohort sizes to combat this variance. Indeed, such variance in the levels of α-synuclein pathology is commonly reported in PD (Jellinger, 2011; Tong et al., 2010).

5.1.2 Cognitive and behavioural characteristics of the study cohort

The distribution and frequencies of the behavioural data present less controversy than the pathology data. Hallucinations are typically associated with the Lewy body dementias and not AD (Bjoerke-Bertheussen et al., 2012; McKeith et al., 2005). Perhaps the most note-worthy observation is the low prevalence of depression in the DLB cases. Depression is a supportive diagnostic feature of DLB (McKeith et al., 2005), and a number of studies have found DLB to be associated with higher incidence of depression than AD (Ballard et al., 1999; Fritze et al., 2011a; Klatka et al., 1996; Yamane et al., 2011). Fritze and colleagues combined DLB and PDD into a single group when making their comparison to AD, the PDD group in this study had relatively high depression scores, so had the same approach been taken a similar finding may have been encountered. Furthermore, not all studies have found DLB to be associated with a higher incident of depression than AD (Rockwell et al., 2000; Samuels et al., 2004). Clearly there is considerable variance in the prevalence of depression in dementia, even without accounting for the difficulty of diagnosis and under-reporting of this symptom.

The profile of cognitive impairment across diagnostic groups was not unusual apart from the lack of AD cases categorised as having MCI, but again this is probably a reflection of the selection criteria for AD cases specifying those with 'pure' AD, and a facet of the autopsy cohort, as few individuals die at an MCI stage and even less come to autopsy.

Rockwell and colleagues found a higher incident of depression in DLB cases with a greater severity of cognitive impairment (Rockwell et al., 2000), that this was not replicated in this cohort is perhaps indicative of the relatively lower percentage of DLB cases in the severely impaired cognition category compared to AD.

5.2 Characteristics of the synaptic biology, according to clinical diagnosis and brain region.

Another key aspect of the hypotheses was the determination of any differences in synaptic biology between diagnostic groups; something of particular importance with regards to delineating DLB and PDD, an outcome which is of relevance to clinicians in addressing neuroleptic sensitivity, and in general to provide assistance in establishing the degree of commonality shared by DLB and PDD.

5.2.1 Changes in β-III-tubulin, PSD95, synaptophysin and ZnT3 are diagnosis and brain region specific

In BA9 there was a reduction of PSD95, SPP and ZNT3 in PDD cases compared to controls – and of PSD95 and ZnT3 in DLB cases; something that was not observed in AD cases. This would suggest there to be a deficit of pre and post-synaptic terminals in the PFC of PDD accompanied by dyshomeostasis of Zn at the synapse, with a similar picture in DLB – excepting the loss of pre-synaptic terminals. In the case of ZnT3, this was corroborated by IHC. It is interesting to note the lack of a deficit in these cellular features in the AD cases. Furthermore, the lack of significant change in Btub values across the diagnostic results suggests the dementia cases are not characterised by any substantial neuronal loss from the PFC.

The creation of residual variables for those proteins that were significantly predicted by either Aβ or tau pathology (in the case of BA9 these were PSD95 and SPP) allowed the effect of this pathology to be statistically negated. That this resulted in no alteration to the aforementioned diagnostic differences in PSD95 or SPP levels, would suggest that AD pathology that is not a dominating mediator of the synaptic dysfunction observed in PDD and DLB prefrontal cortex.

In BA24 there were few significant alterations in the levels of the proteins of interest between diagnostic groups. However, there was a marked increase in PSD95 values in AD cases – something that has been found previously (albeit using immunohistochemistry and on fewer AD cases) (Leuba et al., 2008). In neurodegenerative conditions it is relatively unusually to find an increase in synaptic proteins; however, this may be a compensatory mechanism to alleviate effects of factors not

measured by this study such as synaptic soluble oligomers of the pathologic proteins such as Aβ. Upregulation of PSD95 has been reported in post mortem thalamic tissue from schizophrenic patients (Clinton et al., 2006), which the authors propose to be a compensatory response to NMDA receptor dysfunction.

It is interesting to note that residual values for ZnT3 in BA24 that accounted for the predictive effect of Aβ and tau scores produced no difference in terms of the pattern of changes between diagnostic groups. This would appear to suggest that, whilst AD pathology correlated to ZnT3 values, it is not driving any significant alterations in these values.

AD cases had significantly lower levels of Btub, PSD95 and SPP in BA40. This suggests that there is a substantial loss of neurons and synaptic terminals in BA40 of AD cases compared to control cases – and also to PDD cases, which appear highly similar to controls. DLB cases appeared to follow this trend – although not to a degree that was statistically distinguishable. Strikingly, the creation of residual variables for the tangle score – which had a significant relationship to the values of all proteins in BA40 – completely abolished the substantial reductions in Btub, PSD95 and SPP in AD cases, and the lesser reductions in DLB cases. Thus, a compelling link between tau pathology and synaptic and neuronal loss is established.

5.2.2 Changes in pre and post-synaptic terminal markers relative to changes in the neuronal population, are brain region and diagnosis specific.

Creation of ratios of the pre and post-synaptic markers SPP and PSD95 to Btub allowed changes in these proteins relative to neuronal loss to be approximated, thus allowing changes at the synaptic terminals to be dissected from changes due to neuronal loss. The importance – but not necessity - of both SPP and PSD95 to synaptic function (best demonstrated by knockout mice that survive yet develop cognitive phenotypes (Han and Kim, 2008; Migaud et al., 1998; Schmitt et al., 2009)) allows two deductions to be made about a loss of either protein; that synaptic terminals exist with a reduction in quantity of the protein – and the consequent dysfunction, or that neurons exist with a complete loss of some synaptic terminals – with the remaining terminals containing relatively

normal quantities of the protein in question. In other words, a reduction in SPP to 50% of control levels could correspond to 100% of synapses with 50% SPP levels, or a loss of 50% of synapses – with the remaining 50% containing control levels of SPP. In reality it is likely that a combination of both scenarios occurs plus, as it is not possible to actually quantify synapses by western blotting, this report will relate deficits in SPP or PSD95 to synaptic dysfunction without attempting to elucidate whether this dysfunction is due to synapses with significant reductions in key proteins or neurons with significant reductions in synapses.

In BA9 PDD cases were found to have significant pre-synaptic dysfunction compared to AD cases – and a non-significant trend showing a loss of pre-synaptic marker relative to control and DLB cases. A similar observation can be made with respect to PSD95 – there is a loss of post-synaptic marker in DLB and PDD compared to control and AD cases. This follows the pattern of change in protein values in BA9 and serves to confirm that neuronal loss is not the cause of the reductions seen in the synaptic proteins measured.

There was a lack of any significant differences in pre-synaptic marker (relative to neuronal loss) in BA24 between diagnostic groups, and thus suggesting no significant pre-synaptic dysfunction in dementia cases, confirming what was found for SPP values. However, BA24 in AD cases was characterised by an increased ratio of PSD95 to Btub, suggesting either the control cases (in addition to DLB and PDD) had deficits at the post-synaptic terminal or that AD cases had compensatory upregulation of PSD95 (as described in the preceding section).

The ratios of SPP to Btub and PSD95 to Btub were not significantly different between diagnostic groups. This finding is in contrast to the decreases seen in these proteins in AD cases (see preceding section), but is easily explained by observing that Btub is decreased to a similar extent as SPP and PSD95. Thus it would appear that the synaptic deficits detected in BA40 in AD cases are the result of neuronal loss – evinced by reduced Btub – and not a specific deficit to pre or post-synaptic terminals – demonstrated by the restoration of AD cases to control levels when ratios are used.

5.2.3 Changes in the levels of ZnT3, relative to the synaptic marker synaptophysin, are brain region and diagnosis specific.

In a similar manner to SPP and PSD95 (as discussed in the previous section), changes in ZnT3 levels could be a consequence of a loss of synaptic vesicles - this being the physiological location for ZnT3 (Palmiter et al., 1996), or be independent of any loss of synaptic vesicles. Thus the ratio of ZnT3 to SPP was calculated for the three brain regions where ZnT3 and SPP were quantified to allow the nature of the changes in ZnT3 levels with respect to synaptic vesicles to be determined.

There was a significant decrease in the ratio of ZnT3 to SPP across the three dementias, and which is the most substantial in AD cases. These observations should be compared to those discussed in section 4.1.1, concerning the changes in protein values in BA9 according to diagnosis. Whilst no statistically significant change was found between control and AD cases for either ZnT3 or SPP levels, it is clear that SPP levels are generally higher in AD cases than controls, with the opposite being the case for ZnT3. Thus, in consideration, it is not so surprising that upon combining the values for these two proteins into a ratio a significant reduction is detected in AD cases compared to controls, additionally, there was a significant increase in the ratio of SPP to Btub in AD cases compared to controls in BA9. Thus it can be suggested that the observed deficit of ZnT3 is not an artefact of synaptic vesicle, synapse of neuronal loss, but has occurred independently. The same argument applies – to a lesser extent – to the reduction in ZnT3 levels seen in DLB and PDD cases.

Furthermore, due to the prediction of the ratio of ZnT3 to SPP by the tangle score in BA9 – a residual variable for the ratio was created to statistically remove this predictive effect of tangles. As a consequence of the above steps, there was no longer any significant difference in the ratio of ZnT3 to SPP between diagnostic groups. Thus it would appear that tangle pathology is a significant contributor to the observed deficits in ZnT3 in BA9.

In BA24 there were no significant differences between diagnostic groups in terms of the ratio of ZnT3 to SPP; suggesting that there was no loss of ZnT3, with respect to synaptic vesicles, in the dementia cases.

Contrastingly, in BA40 there were marked reductions in the ratio of ZnT3 to SPP for all dementias compared to controls – with the largest reduction in DLB cases. Again this serves to highlight the different insight offered by expressing the proteins of interest as ratios, as the greatest deficit for the proteins in BA40 was found in AD cases, yet the ratio of ZnT3 to SPP in BA40 is lowest in DLB cases. This suggests DLB cases are characterised by a loss of ZnT3 from synaptic vesicles in BA40, whereas some of the loss of ZnT3 in BA40 seen in AD cases, whilst not statistically significant as an independent measure, is due to reductions in synaptic vesicles. It should be remarked that this loss of ZnT3 in AD cases is unlikely to be a consequence of neuronal loss – as there was no significant difference in the ratio of SPP to Btub between AD or control cases.

5.3 The relationships of synaptic biology to behavioural and cognitive data.

It was hypothesised that synaptic dysfunction – measured by deficits in the synaptic proteins featured in this study – would underlie cognitive impairment and increased behavioural symptoms in dementia cases.

5.3.1 Associations between ZnT3, depression and cognition in BA9.

ZnT3 values in BA9 predicted the semi-quantitative depression score in an inverse manner, such that cases with higher depression scores had lower ZnT3 levels in BA9. It must be noted that this analysis included control cases under the assumption that they did not have clinical depression, an assumption substantiated by the MRC London Neurodegenerative Diseases Brain bank's criteria for accepting cases as a control – which includes assessment of G.P. notes. Substantial evidence has been accumulated linking Zn in the brain to depression; so whilst the association between Zn dyshomeostasis in the prefrontal cortex and depression reported in this study is in accordance with the literature, care must be taken with the interpretation of the relationship between ZnT3 and depression, in light of the strong relationship between cognitive impairment (disease severity) and depression. The data available to this project did not have sufficient statistical power to distinguish between the relationships cognitive impairment and depression to ZnT3 in BA9.

Multiple links were observed between ZnT3 and cognition in BA9 and BA40, both ZnT3 values and the ratio of ZnT3 to SPP predicted cognitive impairment in BA9 and were significantly reduced even in dementia cases with high MMSE scores (the MCI group – scores of 24 to 30) compared to control cases. Control cases had significantly higher ZnT3 values and ratio of ZnT3 to SPP than all the other cognitive impairment categories yet there were no significant reductions between cases classified as having some degree of cognitive impairment. Thus, it appears from the data that Znt3 is reduced from an early stage of cognitive impairment, or dementia progression. Furthermore, this reduction is not attributable to a loss of synaptic vesicles (evinced by the ratio of ZnT3 to SPP maintaining the

same association to cognitive impairment as ZnT3 values). The comparison of groups by one-way ANOVA would suggest that after an initial decline with very early and/or slight cognitive impairment there are no further significant reductions in Znt3, however the one-way ANOVA test is biased towards large differences between groups and less sensitive to a slight but consistent trend across a range of groups (Coolican, 2009). However, regression analysis posses the opposite bias and so has shown Znt3 values and more importantly, the ratio of ZnT3 to SPP, in BA9, to predict cognitive impairment. This is indicative of a trend of decreasing ZnT3 with increasing cognitive impairment.

A similar, albeit weaker, relationship between ZnT3 and cognition was observed in BA40; where both ZnT3 values and the ratio of ZnT3 to SPP predicted cognitive impairment. That there were no significant differences between the cognitive impairment groups, in spite of the highly significant regression coefficient, reinforces the earlier point about the relative bias of one-way ANOVA and regression analysis.

Thus, it can be concluded that dyshomeostasis of synaptic Zn in BA9 and BA40 contributes to cognitive impairment; and that in BA9 this is from the outset and continues until impairment is severe. These findings are consistent with the reports of cognitive impairment in mice lacking ZnT3 protein (Adlard et al., 2010) which prompted the part of the hypothesis of this study concerning Zn and cognition.

Despite the accumulation of evidence connecting reductions in cortical zinc to depression, there has been no reported investigation into ZnT3 and depression. Adlard and colleagues did not test for behavioural symptoms such as depression in their ZnT3 knockout mice. This is not surprising given the difficulty and controversy of modelling depression in mice, due to a host of factors such as anthropomorphism, lack of biomarkers, universal treatment and heterogeneity of symptoms (Berton et al., 2012). Animal models of depression have demonstrated the importance of zinc in modulating this behaviour. Zinc deprivation has been shown to induce depressive-like behaviour in the forced swim test (Młyniec et al., 2012). However, there may well be significant differences in the etiology of

depression in dementia compared to depression in non-demented individuals, thus it remains a challenge to model this facet of dementia.

In addition to ZnT3; PSD95 values and ratio to Btub were shown to predict cognitive impairment in BA9 but not BA40. However, there were no significant differences between groups, highlighting the more gradual nature of reduction of PSD95. Therefore, the data suggest that a gradual loss of PSD95, in excess of any neuronal loss, contributes to cognitive impairment. In other words, post-synaptic dysfunction, rather than pre-synaptic dysfunction or neuronal loss, is the greater contributor to cognitive decline in the individuals with dementia in this study.

5.3.2 Post-synaptic alterations in the study cohort

Reductions in PSD95 have been reported in the hippocampus of individuals with MCI (Sultana et al., 2010); the authors suggested that, amongst the myriad roles of PSD95 at the synapse, it is the clustering of NMDA receptors and the consequent impact on LTP that may provide the mechanism for a reduction in PSD95 to impact cognition. Observations in animals support a connection between PSD95 reduction and cognitive impairment, in particular the original study in which the PSD95 gene was knocked-out in mice, leading to impaired learning and memory (Migaud et al., 1998). Mice overexpressing human APP develop cognitive deficits (according to the Morris water maze) and were characterised by synaptic dysfunction, including reduced PSD95, in the hippocampus (Simón et al., 2009). A similar finding, reductions in a number of pre and post-synaptic markers including PSD95, was made by Majdi and colleagues in aged cognitively impaired rats (Majdi et al., 2007).

In this study, it was found that PSD95 was significantly reduced (relative to controls) in BA9 from DLB and PDD cases, but not in AD cases. Whereas, in BA24, PSD95 values were increased in AD (relative to controls) and not changed in PDD or DLB; and in BA40 PSD95 values were decreased in AD and unchanged in DLB and PDD. These patterns of change are independent of neuronal loss, apart from the decrease in AD BA40, which appears to be related to neuronal loss and therefore may not represent overt synaptic dysfunction.

PSD95 levels are relatively well characterised in post-mortem AD cortex, due to its predominance as the primary post-synaptic marker; several groups have found reductions in PSD95 in AD cases (Gylys et al., 2004; Love et al., 2006). However, Love et al. only measured PSD95 in the temporal cortex, whilst Gylys and colleagues measured PSD95 in the prefrontal cortex, neither group examined BA24 or BA40 and so these reports are not directly comparable. Additionally it should be remarked that an increase has been reported for PSD95 in AD post-mortem tissue (mentioned in the discussion of SPP) (Leuba et al., 2008).

5.3.3 Alterations in zinc regulation in the study cohort

Loss of ZnT3 reduces the pool of so called 'free' Zn (Zn that is not bound to metalloproteins and is thus available for synaptic release) (Cole et al., 1999) and so it is reasonable to assume some of the described anti-depressant and cognitive effects of Zn will be reduced as a consequence. The more important question is how this reduction in ZnT3 could have arisen.

Lee and colleagues used APOe knockout mice to establish the first link between APOe and ZnT3; the mice exhibited reductions in ZnT3, an associated modulator of ZnT3 – AP3δ – and synaptic Zn (Lee et al., 2010). Whilst it is accepted that APOe binds Zn along with Aβ in plaques, the different isoforms of APOe have widely different affinities for Zn binding; APOe2 has the highest and APOe4 the lowest – which the authors speculate in may leave greater quantities of Zn free to bind Aβ and precipitate aggregation and plaque formation (Lee et al., 2010).

Consistent with this hypothesis is the observation that Zn is preferentially elevated in the brains of individuals with AD who carry the APOe4 allele, to the extent that the authors state raised Zn levels to be an independent risk factor for AD (González et al., 1999). Lee et al. did not propose a mechanism through which APOe might reduce ZnT3 expression, although they confirmed the synaptic health of the mice through SPP quantification and state the need to address the question of how the different Zn binding affinities of the APOe isoforms affects synaptic Zn. AP3 targets ZnT3 to the vesicle membrane (Salazar et al., 2004) and so the reduction in the delta subunit of AP3 found by Lee et al (Lee et al., 2010) suggests APOe may impair the correct targeting and localisation of ZnT3 to the vesicle membrane.

The possible behavioural manifestations of a reduction in synaptic Zn have the potential to be diverse and complicated. Ablation of the ZnT3 protein (and by extension synaptic Zn (Cole et al., 1999)) in mice causes cognitive deficits (Adlard et al., 2010) yet removal of synaptic Zn may also prevent synaptic accumulation of Aβ oligomers; as evinced by studies in Tg2576 APP transgenic mice crossed with ZnT3 knockouts (Lee et al., 2002) and hippocampal slices (either from ZnT3 knockout

mice or with Zn chelators) incubated in soluble Aβ oligomers (Deshpande et al., 2009). Thus these studies appear to suggest synaptic Aβ mediated by Zn is not linked to cognitive impairment. Perhaps these studies could be taken as evidence against the amyloid hypothesis. However Adlard et al offer the theory that synaptic Aβ aggregates deny synaptic Zn to its physiological roles and so this could explain how reductions in synaptic Zn can be consistent with reduced cognition and increased depression, in the presence of Aβ aggregation driven by dyshomeostasis of synaptic Zn.

Additionally, given that Zn can aggregate tau and α-syn (Boom et al., 2009; Mo et al., 2009; Paik et al., 1999; Yamin et al., 2003), it would be interesting to see the effect of Zn removal in a model of either of these pathologies. Compounding the picture are the data suggesting reductions in Zn to be instrumental in depressive and anxiety like behaviours (Szewczyk et al., 2011).

The data presented here supports a link between reduced ZnT3 (and thus reduced synaptic Zn) to both cognitive impairment, and depression, which are consistent with the literature. However, reductions of ZnT3 in the prefrontal cortex in cases with higher Aβ pathology should not be expected if synaptic Zn is precipitating Aβ pathology. Although clearly tau pathology and other factors not measured in this study play a significant role.

Activity-dependent regulation of ZnT3 levels – if Zn is depleted then cellular response is to reduce ZnT3? A compensatory increase in ZnT3 could make more sense. However if AP3 is reduced or dimerisation of ZnT3 affected then an explanation for reduced ZnT3 would have been offered.

It should be remarked that there was no significant link between ZnT3 (and any other of the proteins measured) in BA24 and cognition.

5.3.4 Alterations in the localisation of ZnT3

The ZnT3 immunohistochemistry has revealed two interesting findings; firstly a reduction in synaptic staining in PDD cases, a finding supportive of Western blot data and discussed in the preceding section, and secondly immunolabelling in the nuclear membrane. This latter observation was of interest because ZnT3 has not previously been reported in this location in human cortex. ZnT3 is

expressed predominantly in synaptic vesicles and would thus be expected to be localised to the presynaptic terminal (Palmiter et al., 1996; Salazar et al., 2004); indeed a synaptic pattern of staining was obtained with the subsequent IHC comparing diagnostic groups.

Znt3 has been reported in a perinuclear localisation in human retinal pigment epithelium (RPE) cells (Leung et al., 2008) and in cortical neurons in a mouse model of lysosomal storage disorder (Kantheti et al., 1998; Stoltenberg et al., 2004). Leung and colleagues do not offer comment upon their finding and the relevance of ZnT3 expression in RPE cells to expression in cortical neurons remains to be determined. However, the perinuclear ZnT3 expression in MOCHA mice is suggestive of erroneous ZnT3 trafficking and targeting to vesicles as a consequence of the loss of function of AP3 in these mice, supported by evidence that AP3 directs ZnT3 to vesicles (Salazar et al., 2004). Thus, it is conceivable that a similar outcome (reduced synaptic and increased perinuclear ZnT3) arises in DLB and PDD as a consequence of altered cellular transport and trafficking of ZnT3. AP-3 reductions have been reported in AD human neocortex (Yao and Coleman, 1998) but no investigation has been undertaken in DLB or PDD. It would therefore be interesting to investigate AP3 levels in these dementias to determine if alterations in AP3 underlie any defects in cellular trafficking.

There remain other explanations for this perinuclear ZnT3 staining. Clearly, the ZnT3 protein is manufactured within the cytoplasm, and so it could be that the staining reflects ZnT3 awaiting transport to vesicles and the synapse. It is also possible that the antibody is detecting another member or members of the ZnT family, due to the high level of similarity in the sequence between ZnT proteins. ZnT3 and ZnT9 have been reported to be associated with the nuclear membrane under certain conditions, including high Zn concentrations and hormone exposure (Huang and Tepaamorndech, 2013). However the specificity of ZnT3 Western blotting casts doubt on this as an explanation. Ultimately, the failure to replicate the perinuclear Znt3 staining, (stemming from the lack of time available for a comprehensive investigation of ZnT3 staining in multiple brain regions

from a large number of cases), combined with the conflicting and sparse literature on ZnT3 localisation, makes it difficult to fully attribute an explanation.

5.3.5 Alterations in the pre-synaptic density in the study cohort

Since the first investigations into synaptic density, and the consequent verification of the hypothesis that a decrease in synaptic density and synapse loss correlates to the severity of cognitive deficit, synaptophysin has been consistently found to be decreased in the PFC in AD compared to non-demented control cases (Clare et al., 2010; Davies et al., 1987; Leuba et al., 2008; Minger et al., 2001; Terry et al., 1991). That this study found no significant difference in SPP levels between AD and controls in BA9 is unexpected and clearly not congruent with the majority of the literature. However SPP levels in BA40 were significantly decreased in AD, which was expected given the severity of AD pathology in this region.

In light of this the first recourse was to the validity of the experiments conducted for this study. The loading of the correct volume per sample (and hence protein concentration) appears consistent as beta-II-tubulin was immunoblotted and quantified on the same membranes – and in tandem with SPP – and was not increased relative to controls in BA9. Furthermore, BA9 was not analysed in isolation but alongside (on the same gels as) BA24 and BA40, and so errors in protein transfer or treatment of membranes cannot explain the specificity of the unexpected results to one brain region - BA9.

In examining the literature on SPP and other synaptic proteins some inconsistencies have been found. Leuba et al. reported an increase in PSD95 in post-mortem AD versus control tissue (from BA9), which was accompanied by a decrease in SPP, and offered compensatory upregulation of PSD95 as a response to decreased pre-synaptic activity as an explanation (Leuba et al., 2008). Interestingly the opposite was observed in the ZnT3 knockout mice; SPP was increased whilst PSD95 was decreased after 6 months – the time point at which cognitive deficits were observed (Adlard et

al., 2010). The authors also suggest this to be a compensatory mechanism involving reductions in ZnT3 affecting Zn homeostasis – which has a knock on effect at the post-synaptic terminal. Thus it is plausible that the pre and post-synaptic terminals are involved in a reciprocal feedback mechanism governing upregulation of key proteins as a response to deficits in other proteins.

Additional evidence of relevance here, comes from a study on post mortem tissue from patients with progressive supranuclear palsy, in which SPP levels in the frontal cortex were reported to be elevated in demented subjects compared to non-demented (Bigio et al., 2001). Despite the extremely low number of cases (n=8), this study – along with the other observations outlined above - serves to highlight the complexities inherent in linking any single element of the vast synaptic machinery too closely to cognition.

With respect to the number of cases – it should be noted that this study reported SPP values for BA9 from 16 AD cases, a similar number to many of the referenced studies on SPP. There are other case specific differences between this study and literature reports, for example in the severity of cognitive deficit in the AD cases. When Minger et al. grouped their AD cases by severity of cognitive deficit based upon MMSE score – only cases with a score of 2 or less had significantly reduced PFC SPP levels (Minger et al., 2001). In contrast, 7 of the 16 AD cases in this study had MMSE scores of 13 or higher, making it perhaps less surprising that SPP levels were relatively preserved. However SPP has also been reported to be lost as an early event with respect to the time course of cognitive decline, a contradiction that likely reflects differences in the method and sensitivity of cognitive assessment (Masliah et al., 2001a).

A final explanation for the inexplicable brain region specificity seen in the preservation of SPP levels was arrived at through analysis of the length of time samples spent in storage at -80°C. A strong correlation and predictive effect was detected for time in storage solely for SPP in BA9. This was expected for the AD cases as this group were all recent additions to the brain bank (within the last 4 years), whereas the other 3 diagnostic groups included cases dating from the late 1980s. What was

striking was that only BA9 SPP values, and no other brain region or protein, was affected by the time in storage.

Increased duration of frozen storage undergone by brain tissue has been shown to relate to reductions in muscarinc receptors in several brain regions, although the authors do not speculate on the mechanism(s) for this observation, but merely caution researchers to consider frozen storage time as a confounding variable (Rodŕiguez-Puertas et al., 1996).

A similar finding concerns brain region specific differences in the effect of PMD on synaptic protein levels. Siew and colleagues found PSD95 and syntaxin to be affected (decreased) by an increase in PMD in BA9 but not BA21, and suggested that the lower synaptic density found naturally in BA9 may somehow cause greater exposure of synaptic proteins to degradative processes than occurs in other brain regions (Siew et al., 2004). Whilst Siew and colleagues did not detect changes in SPP related to PMD of up to 72 hours, due to the stability of SPP arising from its secure embedding into the vesicle membrane, it is conceivable that great lengths of time in storage at -80°C could disrupt the lipid environment of the vesicle membrane and that this would be compounded by the reduced synaptic density of BA9. Certainly disruption to the lipid membrane of vesicles during freezing has been reported (Strauss and Hauser, 1986), albeit without investigation into the effect of this on membrane embedded proteins – so any resultant change to SPP levels remains conjecture.

It is perhaps noteworthy that BA9 is in greater demand from brain banks for research samples than the other regions, thus it could be speculated that the coronal slices containing BA9 had been subjected to more thawing and re-freezing over the years as samples were taken. That this could compound any damaging effect of ice crystals to membranes is not beyond possibility – see the methods section for the drebrin ELSIA.

Interestingly, the solubility of SPP has been reported to be altered by Aβ42 peptides in APP double transgenic mice (hAPPsw/hPS2m). These mice had higher concentrations of Aβ42 than the other

transgenics examined, and a reduction in soluble SPP but not total SPP. The authors pursued this investigation as a response to conflicting accounts of SPP levels both increased and decreased in mouse models of AD, and thus propose this alteration of SPP solubility as an explanation for the apparent changes in SPP levels (Hwang et al., 2011). This would appear to be supported by observations that SPP is scarce at sites where Aβ oligomers have accumulated (Ishibashi et al., 2006), however this observation was based upon IHC, which was the same method used by Hwang et al. to determine total SPP. It is therefore more likely that this is due to oligomer and/or human specific effects on SPP rather than that of Aβ42.

Additionally, mediation, in particular anti-psychotics and anti-depressants, have been reported to induce changes in synaptic proteins when administered to rats, including an increase in SPP (Varea et al., 2007) (Eastwood et al., 1997). However this is not consistent with findings by Gabriel and colleagues that SPP levels in post-mortem tissue were not different between schizophrenia patients that had or had not taken anti-psychotics (Gabriel et al., 1997). Unfortunately, due to a lack of medication data, it was not possible to determine if there was any effect of medication on SPP levels in this study.

5.3.6 Associations between synaptic biology in BA40 and hallucinations

It was found that both α-synuclein pathology and SPP levels, in the parietal cortex, predicted hallucinations. The parietal cortex has an established role in visual processing (see introduction section on parietal cortex for details). Furthermore, it has been suggested that the neural basis for hallucinations resides in 'higher' cortical regions, such as the parietal cortex, as opposed to the visual cortex; a theory based upon observations from fMRI studies showing intact visual cortex in DLB patients (Kenny et al., 2012).

α-syn pathology has previously been linked to hallucinations (Harding et al., 2002), although this pathology was in the inferior temporal cortex. Harding and colleagues did not find a relationship between parietal α-syn pathology and hallucinations; but remark upon the ability of subtle

differences in the precise brain region(s) selected, in addition to the inherent cohort differences, to affect findings of this nature, in defence of a study previous to theirs that found no relationship between pathology and hallucinations in DLB whatsoever (Gómez-Isla et al., 1999). It is therefore relevant to remark that Harding and colleagues scored α-syn pathology in BA39 and not BA40 and compiled staining from ubiquitin and α-syn antibodies to give a LB count rather than a semi-quantitative score encompassing all types of α-syn pathology (an approach established to be better at comparisons between staining techniques and laboratories (Compta et al., 2011), as was done in this study). Additionally, a striking cohort-specific difference between Harding et al. and this study is the paucity of tau pathology in the DLB cases utilised by Harding, an observation in direct contrast to the DLB cases in this study.

Harding and colleagues highlight the link between their findings and the reported association between deficits in cholinergic neurotransmission in the temporal cortex and visual hallucinations (Ballard et al., 2000; Marra et al., 2012; Perry et al., 1990), and suggest a need for further work to establish the sequence of these events with an aim of establishing whether the α-syn pathology propagates synaptic dysfunction and loss of cholinergic neurotransmission, or if it is caused by this cholinergic deficit.

A study by Papapetropoulos and colleagues was the only report of a connection between α-syn pathology in the parietal cortex and hallucinations found in a literature search using pubmed.com (Papapetropoulos et al., 2006). The authors used BA39 to represent the parietal cortex and made the comparison between PD cases with and without a history of hallucinations, the consequence of which was a significant association between LBs in several brain regions (including the amygdale, temporal cortex and parietal cortex) and visual hallucinations. This corroborated an earlier study that found decreased metabolic activity (according to FDG-PET) in the parietal cortex (including BA40) of PD patients with visual hallucinations, despite the exclusion of individuals meeting criteria for dementia (Nagano-Saito et al., 2004).

To the author's knowledge, there are no publications reporting a direct connection between SPP and hallucinations. However, schizophrenia research has long focussed on reported links between synaptic plasticity and hallucinations, albeit generally of an auditory nature (Stephan et al., 2009). Port and Seybold suggest that potentiated LTP in the hippocampus forms the biological basis for psychosis and hallucinations in schizophrenia (Port and Seybold, 1995), and that blockade of NMDA receptors may alleviate this. It is plausible that such an increase in LTP could involve changes to synaptophysin levels as SPP is an established regulator of synaptic plasticity (Alder et al., 1995). There is considerable evidence for SPP playing a role in schizophrenia and hallucinations, the majority of studies have found SPP expression to be reduced in schizophrenia (summarised by Shen et al.) (Shen et al., 2012); however, elevated levels of SPP have been reported in the cingulate gyrus of schizophrenia patients (Gabriel et al., 1997), as has increased synaptic density (Aganova and Uranova, 1992; Selemon et al., 1995).

As discussed in the preceding SPP section, a number of confounding factors could cause an artificial increase in synaptic proteins such as synaptophysin, amongst which, the reports of anti-psychotics and anti-depressants producing elevated SPP concentrations are the most relevant for consideration here. Nevertheless, hallucinations can be thought of as a gain of function as opposed to a loss of function, thus it is not altogether surprising that they may be accompanied by abnormal increases in neuronal connectivity and synaptic activity.

In summary, this study has established a link between the parietal cortex and hallucinations; the proposed mechanism of which being synaptic dysfunction, primarily mediated by increased Ayn pathology. That elevated SPP was associated with hallucinations is harder to reconcile, despite the precedence for this in schizophrenia, but could be related to medication, or represent a compensatory increase, perhaps in an attempt to correct an imbalance in vesicle dynamics caused by the α-syn pathology. A similar argument stands as to that given by Harding et al. with regards to cause verses consequence of these two events (α-syn and SPP), one which post-mortem studies are severely limited in answering due to the end-stage nature of any observations made. It would have

been interesting to have data on cholinergic neurotransmission in the cortical regions studied in this project to determine if the connection between cholinergic deficits and hallucinations was replicated.

As a final remark, it should be clarified that SPP did not predict hallucinations when including controls because SPP was elevated in control cases, this was coupled with no hallucinations; thus the association between high hallucination scores in PDD and DLB cases with elevated SPP was balanced out.

5.3.7 Rationale for use of cognitive impairment categories based upon MMSE score

The 'cut off' score below which an individual is regarded as having dementia was originally recommend as 24 by the creators of the MMSE, Folstein and colleagues (Folstein et al., 1975). However, there have since been a number of proposed revisions to this guideline based upon observations that in populations with higher educational levels or in the very old (90 years and older) this cut off score of 24 is not adequate. A score of 25 as a cut off point for dementia has been suggested (Ganguli et al., 1990), as has a score of 26 (Monsch et al., 1995) and 27 (Kukull et al., 1994). This latter recommendation was specific to cohorts with a higher than average educational background, something which cannot be ascertained for the cohort presented in this project, and so is of less relevance, but serves to highlight the ongoing debate concerning the interpretation of MMSE scores. A cut off score of 25 was chosen as the upper boundary for this study, based not only upon that suggested by Ganguli et al. but on guidelines published on the Alzheimer's Association website (http://www.alz.org/alzheimers_disease_steps_to_diagnosis.asp). The cut off point of 10 or below is a more widely accepted definition for severe dementia (Boller et al., 2002).

5.4 Associations between biochemistry and pathology in the study cohort.

Given evidence of interactions between pathological and synaptic proteins, a key aspect of this project was to determine what relationships existed between the synaptic proteins of interest and the three principle pathologies of the dementias studied, and whether synaptic deficits were underlying pathological deposition or *vice versa.*

5.4.1 Associations between tau pathology and neuronal biochemistry.

In BA9, tangle score predicted SPP values and that in BA40 the tangle score predicted PSD95 and Btub values, additionally, PSD95 values in BA24 predicted tangle scores. It is interesting to note that PSD95 values were increased with higher tangle scores in both instances mentioned above (the prediction was of a direct nature), whereas SPP values in BA9 and Btub values in BA40 had an inverse relationship to tangle score.

Synapses depend upon axonal transport, a process mediated by the cytoskeleton, for the correct delivery and localisation of synaptic proteins (Alberts et al., 2002). Tau plays an important role in stabilising the cytoskeleton, in part through binding to Btub, and is therefore instrumental (albeit indirectly) in the appropriate transport of proteins along the dendrites (Ballatore et al., 2007; Lee et al., 1989; Santacruz et al., 2005). Furthermore, tau pathology has been suggested to be more neurotoxic than Aβ pathology, based upon evidence that it is a better correlate of neuronal death and cognitive decline (Arriagada et al., 1992; Takashima, 2009).

Decreases in pre-and post-synaptic terminal density have recently been reported in a mouse model of tau pathology (Tg-FDD-Tau, a transgenic combination of human mutant tau and human mutant *BRI* – the gene responsible for familial Danish dementia) (Garringer et al., 2013). In this study it was found that SPP decreased in an age-dependent manner, prior to overt tau pathology, which, according to the authors, was suggestive of an effect of oligomeric 'pre-tangle' tau; and served to corroborate other studies suggesting abnormalities in tau function to be causative of synaptic deficits via an impact on axonal transport (Lasagna-Reeves et al., 2011; Yoshiyama et al., 2007). Thus, the finding in this study that increased tau pathology was associated with, and predicted

reduced SPP and Btub levels is not surprising. That increased PSD95 levels are associated with higher tau scores is unexpected and is due to the elevated PSD95 detected in AD cases, this is discussed under PSD95.

5.4.2 Associations between synaptic biochemistry and Aβ pathology.

Synaptic activity has been implicated in the production of Aβ pathology and the conference of susceptibility to Aβ pathology to specific brain regions (Bero et al., 2011). In a search for molecular markers of this synaptic activity mediated regional vulnerability to Aβ deposition, Shinohara and colleagues found a positive correlation between both PSD95 and SPP and soluble Aβ40 and Aβ42 (Shinohara et al., 2013). The authors propose this relationship to be indicative of a causative link between synaptic activity and generation of Aβ. Thus, there are two potentially conflicting theories with regards to synapses and pathology; one being that synaptic dysfunction and loss occurs in dementia as a result of pathological damage from Aβ (Lesné et al., 2006; Shankar et al., 2008; Walsh et al., 2002; Walsh and Selkoe, 2004) and the second that synaptic activity promotes pathological deposition of Aβ (Bero et al., 2011; Cirrito et al., 2005). Possibly these theories can be reconciled with the notion that synaptic activity generates the initial stages of pathological deposition, which eventually reaches toxic levels and damages synapses.

This concept is consistent with the data in this project, where both positive and negative relationships have been found between synapses and pathology. In BA24, PSD95 was elevated, and ZnT3 decreased, in cases with higher Aβ pathology; whereas in BA40, SPP and PSD95 were decreased in cases with higher levels of tau pathology.

5.4.3 Associations between ZnT3 and pathology.

It was found that a loss of ZnT3 from synaptic vesicles predicted plaque and tangle scores in BA9 and α-synuclein scores in BA40. Additionally, in BA24, ZnT3 values (but not the ratio to SPP) predicted plaque and tangle pathology. This latter point is probably due to the higher variation in SPP values in BA24 removing any significance in the ratio of ZnT3 to SPP. The association between ZnT3 and AD pathology, and not to α-synuclein pathology, in BA9, is probably a consequence of the relative absence of BA9 α-synuclein pathology in the PDD cases, coupled with relatively higher AD pathology and reduced ZnT3 levels. Likewise, the lack of association between ZnT3 and AD pathology in BA40 is

a result of the relatively unchanged ZnT3 levels between the cases with the highest AD pathology (AD cases) and the lowest (controls).

As mentioned in the introduction section on ZnT3, a reduction in ZnT3 can be directly equated to a loss of regulation of synaptic Zn as it is the sole vesicular Zn transporter, thus Zn is unable to be released from vesicles as a co-transmitter in the absence of ZnT3; indeed, this is well established in ZnT3 knockout mice (Adlard et al., 2010; Cole et al., 1999; Linkous et al., 2008). The initial reports on the cognitive phenotype of ZnT3 knockout mice (Cole et al., 1999) were confounded by the unexpected preservation of learning and memory in these mice; an unexpected finding in the light of the essential nature of Zn as a modulator of neurotransmission. However, Adlard and colleagues reported the hypothesised cognitive phenotype did occur, but later in life, and proposed an interaction with AD pathology to explain this delayed onset of cognitive impairment in the absence of ZnT3. The mechanism of which, they suggest to be an entrapment of Zn by the lifetime accumulation of plaques producing a similar effect to the ablation of ZnT3.

In vitro studies have implicated Zn in the aggregation of Aβ (Bush et al., 1994; Curtain et al., 2001), tau (Boom et al., 2009; Mo et al., 2009) and α-synuclein (Paik et al., 1999; Yamin et al., 2003). Therefore, the finding of associations between dyshomeostasis of synaptic Zn and these pathological proteins in this study is in accordance with the reported *in vitro* evidence, and the theory proposed by Adlard et al. may offer a connection between this association of Zn and pathology to that observed between Zn and cognitive decline. In summary, it can be hypothesised that loss of regulation of synaptic Zn denies Zn as a modulator of neurotransmission and cognition, and may increase availability of Zn to promote aggregation of Aβ, tau and α-synuclein at the synapse; an event which is likely to further degrade synaptic function and cognition in a positive feedback manner.

5.5 Modulation of synaptic zinc as an approach to disease modification

The published evidence (as discussed in the preceding sections, and supported by the findings of this project) suggest there to be a two-fold story to zinc in dementia; excess Zn can increase pathological proteins whilst its absence is detrimental to cognition and depression. This evidence for an interaction between Zn ions and Aβ pathology that was deleterious to cognition led to investigations into pharmacological modulation of Zn as a potential avenue of disease treatment; with the important caveat that reducing Zn concentrations could worsen cognition even if it reduced the propagation of pathology (especially given the doubt concerning the direct impact of Aβ pathology on cognition) and that increasing Zn concentration could have the reverse effect (Relkin, 2008).

A quinoline derivative, PBT2, has indeed been demonstrated to reduce the interaction between Zn and Aβ whilst increasing the availability of Zn to the synapse for physiological functions (Adlard et al., 2008). PBT2 is classed as an ionophore, a compound able to promote the movement of an ion (in this case Zn) across a cell membrane (Adlard et al., 2008). *In vitro* studies by Adlard et al. have established PBT2 to reduce Zn binding to (and precipitation of) Aβ, and in doing so to mitigate Aβ mediated synaptotoxicity (a finding corroborated by increased SPP levels in APP transgenic mice treated with PBT2). Additionally the authors demonstrated PBT2 to lower concentrations of soluble and insoluble Aβ and plaque scores in Tg2576 APP transgenic mice without affecting overall Zn levels in the brain. Strikingly, these mice displayed improved learning and memory after PBT2 administration that was superior to wild type mice, even wild type that had also been given PBT2.

PBT2 treated APP/PS1 transgenic mice were found to have reduced phospho-tau concentrations, and the Tg2576 mice reduced total tau concentrations (Adlard et al., 2008), although the authors did not speculate on whether this was related to the reductions in Aβ or direct interference of Zn mediated tau aggregation (Boom et al., 2009; Mo et al., 2009). Adlard and colleagues propose PBT2 to elucidate these effects by promoting Zn transport into the pre-synaptic terminals of neurons, thus

denying Zn any role in Aβ aggregation and increasing Zn availability for release with neurotransmitter in its physiological role.

PBT2 has been demonstrated to be well tolerated in a phase IIa clinical trial in patients with mild AD (Lannfelt et al., 2008). Reductions in CSF Aβ42 concentrations were reported in this trial, in addition to hints at an efficacious impact on cognition, despite the trial not being designed to detect this. Subsequent analysis attributed greater statistical significance to the improvement in cognition caused by PBT2 (Faux et al., 2010), leading the authors to conclude that PBT2 was likely to have a rapid beneficial effect on cognition in patients with AD through a disease modifying mechanism, and that further large scale trials were justified.

Despite reports of Zn promoting aggregation of α-syn (Golts et al., 2002; Paik et al., 1999), little investigation has been undertaken with regards to modifying this phenomenon. There has been recent criticism of this theory by Valiente-Gabioud and colleagues (Valiente-Gabioud et al., 2012), who (in light of the fact that Zn is mainly shown to promote α-syn aggregation at mM concentrations, combined with their findings that the Zn-α-syn interaction is low affinity) suggest doubt as to the physiological relevance of Zn-α-syn interactions given that the average brain concentration of Zn falls in the μM range (Takeda, 2000). However, Zn concentrations have been reported in excess of 1mM in synaptic vesicles (Frederickson et al., 2000); so given that synapses are proposed to be the site of α-syn aggregation mediated toxicity and ZnT3 mediated dyshomeostasis (and therefore the location where any Zn-α-syn interaction would have the greatest relevance) it is plausible that Zn can reach concentrations at the synapse that are more than sufficient for promotion of aggregation of α-syn. It would be of interest to explore the potential for modifying the interaction of Zn and α-syn in an *in vitro* model using PBT2.

5.6 Unexpected findings

It could have been expected that some relationships would be detected between synaptic dysfunction and persecution and agitation. However, the relatively low number of cases with scores for these symptoms, and in particular high scores will have impaired the ability to detect any relationship to the biochemistry.

Additionally, it is interesting that there were so few links between α-synuclein pathology and synaptic biochemistry. Only ZnT3 in BA40 and drebrin in BA9 were altered in cases with higher α-syn scores. Several factors could explain this, PDD cases had low levels of α-syn pathology, whilst they did have the higher deficits in synaptic proteins in BA9; this was not sufficiently different to DLB to cause a positive correlation.

It may be that soluble α-syn oligomers are the synapto-toxic species of α-syn and thus have greater responsibility for synaptic deficits. A growing body of evidence supports the synaptic presence and toxicity of such species (Kramer and Schulz-Schaeffer, 2007; Nakata et al., 2012; Tanji et al., 2010). Although the Novacastra antibody used for the majority of α-syn IHC and semi-quantification in this study reveals some extent of these synaptic aggregates, it does not detect soluble species of α-syn. Additionally, as the PET blot developed by Kramer and Schulz-Schaeffer (Kramer and Schulz-Schaeffer, 2007) could not be replicated in our lab (see method section on PET blotting), the extent of the probable contribution of soluble α-syn oligomers to synaptic dysfunction cannot be assessed.

5.7 Concluding Remarks

The principal findings of this study were; the association between reduced ZnT3 in the prefrontal cortex and both cognitive decline and depression, the associations between ZnT3 and alpha-synuclein pathology in the parietal cortex and Aβ and tau pathology in the prefrontal cortex and the differences in synaptic biochemistry between DLB and PDD cases.

Once differences between diagnostic groups were detected it was perhaps inevitable that associations between the proteins of interest and both clinical and pathological data would also be detected because it is this clinical and pathological data that forms the essence of the diagnosis. However it could not be assumed to be guaranteed as the quality and quantity of clinical and pathological data, with particular concern to the control cases, would determine the ability of the statistical methods employed to detect any associations between the biochemistry and the clinical and pathological data. If the control cases had either poor data in terms of missing data, or were poorly selected as controls (i.e. had high pathology) then it would have been immensely harder to detect any possible association between the biochemistry and pathology. This point is especially valid for the behavioural and cognitive data as this data relies upon the selection criteria for control cases due to the assumptions made that control cases fall into the 'absent' or 'none' categories.

It is encouraging to have discovered potential correlates of cognitive decline that are of a synaptic and functional nature as this is consistent with current opinion. Klucken and colleagues have proposed the lack of overt neuronal loss in DLB (compared to AD and controls) (Buldyrev et al., 2000; Lippa et al., 1994) in combination with poor correlation between α-syn pathology and cognitive decline (Colosimo et al., 2003) to support evidence that functional synaptic deficits underlie cognitive impairment in DLB (Klucken et al., 2006; Klucken et al., 2003).

Determining whether biochemical differences exist between PDD and DLB could be of assistance in diagnosis and management of these dementias, particularly in light of neuroleptic sensitivity in DLB patients (Aarsland et al., 2005a; McKeith et al., 1992). CSF is the most likely medium for any

biomarker based upon synaptic proteins. It has been reported that SPP is not sufficiently stable in CSF (due to its high hydrophobicity) to allow accurate or reliable detection, but other synaptic proteins such as SNPA25 and synaptotagmin are (Davidsson et al., 1999), and so if synaptic dysfunction is a point of distinction between DLB and PDD it could be useful to investigate other synaptic markers in CSF to establish the viability of this approach as a biomarker. There are no reports of the viability of detection of PSD95 or ZnT3 in CSF, thus development of an assay to detect these proteins could provide an avenue for a biomarker. However, the differences in synaptic proteins between dementias shown in this project are brain region specific and thus may not be reflected in CSF; this would need to be determined.

The sample size of this study was limited by the availability of DLB and PDD cases with detailed clinical data, and whilst the numbers of these cases was large relative to similar studies on DLB and PDD, they are low in comparison to post-mortem studies of AD and other types of studies. This creates common problems encountered throughout biomedical research when sample sizes are limited, and is a specific problem to post-mortem studies. Button and colleagues recently discussed some of the issues faced by studies with small sample sizes (Button et al., 2013). Amongst these was a bias towards reporting false negatives and an exaggeration of any true effect that is detected. Whilst there was little that could have been done to counter these limitations in this study, the authors also comment that large sample numbers have drawbacks such as the inclusion of poorer quality cases/data in order to improve the n number, something that was addressed by this study.

Finally, it should be remarked that high variance is expected in human studies of this nature, due to the large genetic and environmental heterogeneity inherent in cohorts sourced from such a wide period of time and geographical area.

6 Bibliography

(1997). Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Neurobiol Aging *18*, S1-2.
Aarsland, D., Andersen, K., Larsen, J.P., Lolk, A., and Kragh-Sørensen, P. (2003). Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. Arch Neurol *60*, 387-392.
Aarsland, D., Ballard, C., Walker, Z., Bostrom, F., Alves, G., Kossakowski, K., Leroi, I., Pozo-Rodriguez, F., Minthon, L., and Londos, E. (2009). Memantine in patients with Parkinson's disease dementia or dementia with Lewy bodies: a double-blind, placebo-controlled, multicentre trial. Lancet Neurol *8*, 613-618.
Aarsland, D., Ballard, C.G., and Halliday, G. (2004). Are Parkinson's disease with dementia and dementia with Lewy bodies the same entity? J Geriatr Psychiatry Neurol *17*, 137-145.
Aarsland, D., Perry, R., Larsen, J.P., McKeith, I.G., O'Brien, J.T., Perry, E.K., Burn, D., and Ballard, C.G. (2005a). Neuroleptic sensitivity in Parkinson's disease and parkinsonian dementias. J Clin Psychiatry *66*, 633-637.
Aarsland, D., Rongve, A., Nore, S.P., Skogseth, R., Skulstad, S., Ehrt, U., Hoprekstad, D., and Ballard, C. (2008). Frequency and case identification of dementia with Lewy bodies using the revised consensus criteria. DementGeriatrCogn Disord *26*, 445-452.
Aarsland, D., Sharp, S., and Ballard, C. (2005b). Psychiatric and behavioral symptoms in Alzheimer's disease and other dementias: etiology and management. Curr Neurol Neurosci Rep *5*, 345-354.
Aarsland, D., Zaccai, J., and Brayne, C. (2005c). A systematic review of prevalence studies of dementia in Parkinson's disease. Mov Disord *20*, 1255-1263.
Abbott, J.J., Howlett, D.R., Francis, P.T., and Williams, R.J. (2008). Abeta(1-42) modulation of Akt phosphorylation via alpha7 nAChR and NMDA receptors. Neurobiol Aging *29*, 992-1001.
Abeliovich, A., Schmitz, Y., Farinas, I., Choi-Lundberg, D., Ho, W.H., Castillo, P.E., Shinsky, N., Verdugo, J.M., Armanini, M., Ryan, A.*, et al.* (2000). Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron *25*, 239-252.
Adlard, P.A., Cherny, R.A., Finkelstein, D.I., Gautier, E., Robb, E., Cortes, M., Volitakis, I., Liu, X., Smith, J.P., Perez, K.*, et al.* (2008). Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Abeta. Neuron *59*, 43-55.
Adlard, P.A., Parncutt, J.M., Finkelstein, D.I., and Bush, A.I. (2010). Cognitive Loss in Zinc Transporter-3 Knock-Out Mice: A Phenocopy for the Synaptic and Memory Deficits of Alzheimer's Disease? Journal of Neuroscience *30*, 1631-1636.
Aganova, E.A., and Uranova, N.A. (1992). Morphometric analysis of synaptic contacts in the anterior limbic cortex in the endogenous psychoses. Neurosci Behav Physiol *22*, 59-65.
Aho, L., Parkkinen, L., Pirttila, T., and Alafuzoff, I. (2008). Systematic appraisal using immunohistochemistry of brain pathology in aged and demented subjects. Dement Geriatr Cogn Disord *25*, 423-432.
Aho, L., Pikkarainen, M., Hiltunen, M., Leinonen, V., and Alafuzoff, I. (2010). Immunohistochemical visualization of amyloid-beta protein precursor and amyloid-beta in extra- and intracellular compartments in the human brain. J Alzheimers Dis *20*, 1015-1028.
Akasofu, S., Kosasa, T., Kimura, M., and Kubota, A. (2003). Protective effect of donepezil in a primary culture of rat cortical neurons exposed to oxygen-glucose deprivation. Eur J Pharmacol *472*, 57-63.
Al-Sarraj, S., King, A., Troakes, C., Smith, B., Maekawa, S., Bodi, I., Rogelj, B., Al-Chalabi, A., Hortobágyi, T., and Shaw, C.E. (2011). p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol *122*, 691-702.

Alafuzoff, I., Arzberger, T., Al-Sarraj, S., Bodi, I., Bogdanovic, N., Braak, H., Bugiani, O., Del-Tredici, K., Ferrer, I., Gelpi, E.*, et al.* (2008). Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol *18*, 484-496.
Alafuzoff, I., Gelpi, E., Al-Sarraj, S., Arzberger, T., Attems, J., Bodi, I., Bogdanovic, N., Budka, H., Bugiani, O., Englund, E.*, et al.* (2012). The need to unify neuropathological assessments of vascular alterations in the ageing brain: Multicentre survey by the BrainNet Europe consortium. Exp Gerontol *47*, 825-833.
Alafuzoff, I., Ince, P.G., Arzberger, T., Al-Sarraj, S., Bell, J., Bodi, I., Bogdanovic, N., Bugiani, O., Ferrer, I., Gelpi, E.*, et al.* (2009). Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. Acta Neuropathol *117*, 635-652.
Alafuzoff, I., Pikkarainen, M., Al-Sarraj, S., Arzberger, T., Bell, J., Bodi, I., Bogdanovic, N., Budka, H., Bugiani, O., Ferrer, I.*, et al.* (2006). Interlaboratory comparison of assessments of Alzheimer disease-related lesions: a study of the BrainNet Europe Consortium. J Neuropathol Exp Neurol *65*, 740-757.
Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). Molecular Biology of The Cell, 4th edn (Garland Science, Taylor & Francis Group, New York).
Alder, J., Kanki, H., Valtorta, F., Greengard, P., and Poo, M.M. (1995). Overexpression of synaptophysin enhances neurotransmitter secretion at Xenopus neuromuscular synapses. J Neurosci *15*, 511-519.
Arai, Y., Yamazaki, M., Mori, O., Muramatsu, H., Asano, G., and Katayama, Y. (2001). Alpha-synuclein-positive structures in cases with sporadic Alzheimer's disease: morphology and its relationship to tau aggregation. Brain Res *888*, 287-296.
Arriagada, P.V., Growdon, J.H., Hedley-Whyte, E.T., and Hyman, B.T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology *42*, 631-639.
Attems, J., and Jellinger, K.A. (2008). The dorsal motor nucleus of the vagus is not an obligatory trigger site of Parkinson's disease. Neuropathol Appl Neurobiol *34*, 466-467.
Attems, J., Jellinger, K.A., and Lintner, F. (2005). Alzheimer's disease pathology influences severity and topographical distribution of cerebral amyloid angiopathy. Acta neuropathologica *110*, 222-231.
Atwood, C.S., Bishop, G.M., Perry, G., and Smith, M.A. (2002). Amyloid-beta: a vascular sealant that protects against hemorrhage? J Neurosci Res *70*, 356.
Auluck, P.K., Caraveo, G., and Lindquist, S. (2010). alpha-Synuclein: membrane interactions and toxicity in Parkinson's disease. Annu Rev Cell Dev Biol *26*, 211-233.
Bak, T.H., and Mioshi, E. (2007). A cognitive bedside assessment beyond the MMSE: the Addenbrooke's Cognitive Examination. Pract Neurol *7*, 245-249.
Ballard, C., Corbett, A., and Sharp, S. (2011a). Aligning the evidence with practice: NICE guidelines for drug treatment of Alzheimer's disease. Expert Rev Neurother *11*, 327-329.
Ballard, C., Gauthier, S., Corbett, A., Brayne, C., Aarsland, D., and Jones, E. (2011b). Alzheimer's disease. Lancet *377*, 1019-1031.
Ballard, C., Holmes, C., McKeith, I., Neill, D., O'Brien, J., Cairns, N., Lantos, P., Perry, E., Ince, P., and Perry, R. (1999). Psychiatric morbidity in dementia with Lewy bodies: a prospective clinical and neuropathological comparative study with Alzheimer's disease. Am J Psychiatry *156*, 1039-1045.
Ballard, C., and Howard, R. (2006). Neuroleptic drugs in dementia: benefits and harm. Nat Rev Neurosci *7*, 492-500.
Ballard, C., O'Brien, J., Coope, B., Fairbairn, A., Abid, F., and Wilcock, G. (1997). A prospective study of psychotic symptoms in dementia sufferers: psychosis in dementia. Int Psychogeriatr *9*, 57-64.
Ballard, C., Piggott, M., Johnson, M., Cairns, N., Perry, R., McKeith, I., Jaros, E., O'Brien, J., Holmes, C., and Perry, E. (2000). Delusions associated with elevated muscarinic binding in dementia with Lewy bodies. Ann Neurol *48*, 868-876.
Ballard, C.G., Gauthier, S., Cummings, J.L., Brodaty, H., Grossberg, G.T., Robert, P., and Lyketsos, C.G. (2009). Management of agitation and aggression associated with Alzheimer disease. Nat Rev Neurol *5*, 245-255.

Ballatore, C., Lee, V.M., and Trojanowski, J.Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci *8*, 663-672.
Ballmaier, M., Narr, K.L., Toga, A.W., Elderkin-Thompson, V., Thompson, P.M., Hamilton, L., Haroon, E., Pham, D., Heinz, A., and Kumar, A. (2008). Hippocampal morphology and distinguishing late-onset from early-onset elderly depression. Am J Psychiatry *165*, 229-237.
Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G., Seitelberger, F., Grundke-Iqbal, I., Iqbal, K., and Wisniewski, H.M. (1989). Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. Brain Res *477*, 90-99.
Barnham, K.J., and Bush, A.I. (2008). Metals in Alzheimer's and Parkinson's diseases. Current opinion in chemical biology *12*, 222-228.
Baron, M.K., Boeckers, T.M., Vaida, B., Faham, S., Gingery, M., Sawaya, M.R., Salyer, D., Gundelfinger, E.D., and Bowie, J.U. (2006). An architectural framework that may lie at the core of the postsynaptic density. Science *311*, 531-535.
Barrondo, S., and Sallés, J. (2009). Allosteric modulation of 5-HT(1A) receptors by zinc: Binding studies. Neuropharmacology *56*, 455-462.
Bear, M.F., Connors, B.W., and Paradiso, M.A. (2006). Neuroscience Exploring the Brain, 3rd edn (Lippincott Williams & Wilkins).
Benilova, I., Karran, E., and De Strooper, B. (2012). The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci *15*, 349-357.
Bergem, A.L., Engedal, K., and Kringlen, E. (1997). The role of heredity in late-onset Alzheimer disease and vascular dementia. A twin study. Arch Gen Psychiatry *54*, 264-270.
Bero, A.W., Yan, P., Roh, J.H., Cirrito, J.R., Stewart, F.R., Raichle, M.E., Lee, J.M., and Holtzman, D.M. (2011). Neuronal activity regulates the regional vulnerability to amyloid-β deposition. Nat Neurosci *14*, 750-756.
Berton, O., Hahn, C.G., and Thase, M.E. (2012). Are we getting closer to valid translational models for major depression? Science *338*, 75-79.
Beyer, K., Domingo-Sabat, M., and Ariza, A. (2009a). Molecular pathology of Lewy body diseases. IntJMolSci *10*, 724-745.
Beyer, N., Coulson, D.T., Heggarty, S., Ravid, R., Irvine, G.B., Hellemans, J., and Johnston, J.A. (2009b). ZnT3 mRNA levels are reduced in Alzheimer's disease post-mortem brain. Mol Neurodegener *4*, 53.
Bigio, E.H., Vono, M.B., Satumtira, S., Adamson, J., Sontag, E., Hynan, L.S., White, C.L., Baker, M., and Hutton, M. (2001). Cortical synapse loss in progressive supranuclear palsy. J Neuropathol Exp Neurol *60*, 403-410.
Bjoerke-Bertheussen, J., Ehrt, U., Rongve, A., Ballard, C., and Aarsland, D. (2012). Neuropsychiatric symptoms in mild dementia with lewy bodies and Alzheimer's disease. Dement Geriatr Cogn Disord *34*, 1-6.
Bjorklund, N.L., Reese, L.C., Sadagoparamanujam, V.M., Ghirardi, V., Woltjer, R.L., and Taglialatela, G. (2012). Absence of amyloid β oligomers at the postsynapse and regulated synaptic Zn2+ in cognitively intact aged individuals with Alzheimer's disease neuropathology. Mol Neurodegener *7*, 23.
Blennow, K., Hardy, J., and Zetterberg, H. (2012). The neuropathology and neurobiology of traumatic brain injury. Neuron *76*, 886-899.
Boche, D., Denham, N., Holmes, C., and Nicoll, J.A. (2010). Neuropathology after active Abeta42 immunotherapy: implications for Alzheimer's disease pathogenesis. Acta Neuropathol *120*, 369-384.
Boeve, B.F. (2005). Evidence for cholinesterase-inhibitor therapy for dementia associated with Parkinson's disease. Lancet Neurol *4*, 137-138.
Boeve, B.F., Silber, M.H., and Ferman, T.J. (2004). REM sleep behavior disorder in Parkinson's disease and dementia with Lewy bodies. J Geriatr Psychiatry Neurol *17*, 146-157.
Boller, F., Verny, M., Hugonot-Diener, L., and Saxton, J. (2002). Clinical features and assessment of severe dementia. A review. Eur J Neurol *9*, 125-136.

Bonifati, V. (2008). Recent advances in the genetics of dementia with lewy bodies. Curr Neurol Neurosci Rep *8*, 187-189.
Boom, A., Authelet, M., Dedecker, R., Frédérick, C., Van Heurck, R., Daubie, V., Leroy, K., Pochet, R., and Brion, J.P. (2009). Bimodal modulation of tau protein phosphorylation and conformation by extracellular Zn2+ in human-tau transfected cells. Biochim Biophys Acta *1793*, 1058-1067.
Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol *82*, 239-259.
Braak, H., and Braak, E. (1997). Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol Aging *18*, 351-357.
Braak, H., Del, T.K., Rub, U., de Vos, R.A., Jansen Steur, E.N., and Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. NeurobiolAging *24*, 197-211.
Braak, H., Del Tredici, K., Bratzke, H., Hamm-Clement, J., Sandmann-Keil, D., and Rüb, U. (2002). Staging of the intracerebral inclusion body pathology associated with idiopathic Parkinson's disease (preclinical and clinical stages). J Neurol *249 Suppl 3*, III/1-5.
Bradshaw, J., Saling, M., Hopwood, M., Anderson, V., and Brodtmann, A. (2004). Fluctuating cognition in dementia with Lewy bodies and Alzheimer's disease is qualitatively distinct. J Neurol Neurosurg Psychiatry *75*, 382-387.
Broderick, P.A. (2005). Bioimaging in Neurodegeneration, 1 edn (Totowa: Humana Press Inc.).
Brown, D.F., Risser, R.C., Bigio, E.H., Tripp, P., Stiegler, A., Welch, E., Eagan, K.P., Hladik, C.L., and White, C.L., 3rd (1998). Neocortical synapse density and Braak stage in the Lewy body variant of Alzheimer disease: a comparison with classic Alzheimer disease and normal aging. J Neuropathol Exp Neurol *57*, 955-960.
Brown, D.R. (2010). Oligomeric alpha-synuclein and its role in neuronal death. IUBMB Life *62*, 334-339.
Buckner, R.L., Snyder, A.Z., Shannon, B.J., LaRossa, G., Sachs, R., Fotenos, A.F., Sheline, Y.I., Klunk, W.E., Mathis, C.A., Morris, J.C*., et al.* (2005). Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci *25*, 7709-7717.
Buldyrev, S.V., Cruz, L., Gomez-Isla, T., Gomez-Tortosa, E., Havlin, S., Le, R., Stanley, H.E., Urbanc, B., and Hyman, B.T. (2000). Description of microcolumnar ensembles in association cortex and their disruption in Alzheimer and Lewy body dementias. Proc Natl Acad Sci U S A *97*, 5039-5043.
Burn, D.J., Rowan, E.N., Minett, T., Sanders, J., Myint, P., Richardson, J., Thomas, A., Newby, J., Reid, J., O'Brien, J.T*., et al.* (2003). Extrapyramidal features in Parkinson's disease with and without dementia and dementia with Lewy bodies: A cross-sectional comparative study. Mov Disord *18*, 884-889.
Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M.R., and Sudhof, T.C. (2010). Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science *329*, 1663-1667.
Bush, A.I., Pettingell, W.H., Multhaup, G., d Paradis, M., Vonsattel, J.P., Gusella, J.F., Beyreuther, K., Masters, C.L., and Tanzi, R.E. (1994). Rapid induction of Alzheimer A beta amyloid formation by zinc. Science *265*, 1464-1467.
Buter, T.C., van den, H.A., Matthews, F.E., Larsen, J.P., Brayne, C., and Aarsland, D. (2008). Dementia and survival in Parkinson disease: a 12-year population study. Neurology *70*, 1017-1022.
Butters, M.A., Klunk, W.E., Mathis, C.A., Price, J.C., Ziolko, S.K., Hoge, J.A., Tsopelas, N.D., Lopresti, B.J., Reynolds, C.F., DeKosky, S.T*., et al.* (2008). Imaging Alzheimer pathology in late-life depression with PET and Pittsburgh Compound-B. Alzheimer Dis Assoc Disord *22*, 261-268.
Button, K.S., Ioannidis, J.P., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S., and Munafò, M.R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci *14*, 365-376.
Byers, A.L., and Yaffe, K. (2011). Depression and risk of developing dementia. Nat Rev Neurol *7*, 323-331.

Cagnin, A., Gnoato, F., Jelcic, N., Favaretto, S., Zarantonello, G., Ermani, M., and Dam, M. (2012). Clinical and cognitive correlates of visual hallucinations in dementia with Lewy bodies. J Neurol Neurosurg Psychiatry.
Campbell, T.N., and Choy, F.Y. (2012). Gaucher disease and the synucleinopathies: refining the relationship. Orphanet J Rare Dis *7*, 12.
Caputo, M., Monastero, R., Mariani, E., Santucci, A., Mangialasche, F., Camarda, R., Senin, U., and Mecocci, P. (2008). Neuropsychiatric symptoms in 921 elderly subjects with dementia: a comparison between vascular and neurodegenerative types. Acta Psychiatr Scand *117*, 455-464.
Catani, M., Dell'acqua, F., Bizzi, A., Forkel, S.J., Williams, S.C., Simmons, A., Murphy, D.G., and Thiebaut de Schotten, M. (2012). Beyond cortical localization in clinico-anatomical correlation. Cortex *48*, 1262-1287.
Cato, M.A., Delis, D.C., Abildskov, T.J., and Bigler, E. (2004). Assessing the elusive cognitive deficits associated with ventromedial prefrontal damage: a case of a modern-day Phineas Gage. J Int Neuropsychol Soc *10*, 453-465.
Caviness, J.N., Lue, L., Adler, C.H., and Walker, D.G. (2011). Parkinson's disease dementia and potential therapeutic strategies. CNS Neurosci Ther *17*, 32-44.
Chartier-Harlin, M.C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., Levecque, C., Larvor, L., Andrieux, J., Hulihan, M.*, et al.* (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet *364*, 1167-1169.
Chen, H., and Chan, D.C. (2009). Mitochondrial dynamics--fusion, fission, movement, and mitophagy--in neurodegenerative diseases. Hum Mol Genet *18*, R169-176.
Cirrito, J.R., Kang, J.E., Lee, J., Stewart, F.R., Verges, D.K., Silverio, L.M., Bu, G., Mennerick, S., and Holtzman, D.M. (2008). Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. Neuron *58*, 42-51.
Cirrito, J.R., Yamada, K.A., Finn, M.B., Sloviter, R.S., Bales, K.R., May, P.C., Schoepp, D.D., Paul, S.M., Mennerick, S., and Holtzman, D.M. (2005). Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. Neuron *48*, 913-922.
Claassen, D.O., Josephs, K.A., Ahlskog, J.E., Silber, M.H., Tippmann-Peikert, M., and Boeve, B.F. (2010). REM sleep behavior disorder preceding other aspects of synucleinopathies by up to half a century. Neurology *75*, 494-499.
Clare, R., King, V.G., Wirenfeldt, M., and Vinters, H.V. (2010). Synapse loss in dementias. J Neurosci Res *88*, 2083-2090.
Clinton, S.M., Haroutunian, V., and Meador-Woodruff, J.H. (2006). Up-regulation of NMDA receptor subunit and post-synaptic density protein expression in the thalamus of elderly patients with schizophrenia. J Neurochem *98*, 1114-1125.
Clower, D.M., West, R.A., Lynch, J.C., and Strick, P.L. (2001). The inferior parietal lobule is the target of output from the superior colliculus, hippocampus, and cerebellum. J Neurosci *21*, 6283-6291.
Cohen-Mansfield, J., Marx, M.S., and Rosenthal, A.S. (1989). A description of agitation in a nursing home. J Gerontol *44*, M77-84.
Cole, T.B., Wenzel, H.J., Kafer, K.E., Schwartzkroin, P.A., and Palmiter, R.D. (1999). Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. Proc Natl Acad Sci U S A *96*, 1716-1721.
Colledge, M., Snyder, E.M., Crozier, R.A., Soderling, J.A., Jin, Y., Langeberg, L.K., Lu, H., Bear, M.F., and Scott, J.D. (2003). Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. Neuron *40*, 595-607.
Collerton, D., Burn, D., McKeith, I., and O'Brien, J. (2003). Systematic review and meta-analysis show that dementia with Lewy bodies is a visual-perceptual and attentional-executive dementia. Dement Geriatr Cogn Disord *16*, 229-237.
Collerton, D., Perry, E., and McKeith, I. (2005). Why people see things that are not there: a novel Perception and Attention Deficit model for recurrent complex visual hallucinations. Behav Brain Sci *28*, 737-757; discussion 757-794.

Colloby, S.J., Fenwick, J.D., Williams, E.D., Paling, S.M., Lobotesis, K., Ballard, C., McKeith, I., and O'Brien, J.T. (2002). A comparison of (99m)Tc-HMPAO SPET changes in dementia with Lewy bodies and Alzheimer's disease using statistical parametric mapping. Eur J Nucl Med Mol Imaging *29*, 615-622.
Colosimo, C., Hughes, A.J., Kilford, L., and Lees, A.J. (2003). Lewy body cortical involvement may not always predict dementia in Parkinson's disease. J Neurol Neurosurg Psychiatry *74*, 852-856.
Compta, Y., Parkkinen, L., O'Sullivan, S.S., Vandrovcova, J., Holton, J.L., Collins, C., Lashley, T., Kallis, C., Williams, D.R., de Silva, R.*, et al.* (2011). Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? Brain *134*, 1493-1505.
Cookson, M.R. (2010). The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. Nature reviews Neuroscience *11*, 791-797.
Coolican, H. (2009). Research Methods and Statistics in Psychology, 5th edn (Hodder Education).
Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., and Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science *261*, 921-923.
Cowburn, R.F., Wiehager, B., Trief, E., Li-Li, M., and Sundstrom, E. (1997). Effects of beta-amyloid-(25-35) peptides on radioligand binding to excitatory amino acid receptors and voltage-dependent calcium channels: evidence for a selective affinity for the glutamate and glycine recognition sites of the NMDA receptor. Neurochem Res *22*, 1437-1442.
Cuajungco, M.P., Goldstein, L.E., Nunomura, A., Smith, M.A., Lim, J.T., Atwood, C.S., Huang, X., Farrag, Y.W., Perry, G., and Bush, A.I. (2000). Evidence that the beta-amyloid plaques of Alzheimer's disease represent the redox-silencing and entombment of abeta by zinc. The Journal of biological chemistry *275*, 19439-19442.
Cullen, K.M., Kócsi, Z., and Stone, J. (2006). Microvascular pathology in the aging human brain: evidence that senile plaques are sites of microhaemorrhages. Neurobiol Aging *27*, 1786-1796.
Cullen, V., Sardi, S.P., Ng, J., Xu, Y.H., Sun, Y., Tomlinson, J.J., Kolodziej, P., Kahn, I., Saftig, P., Woulfe, J.*, et al.* (2011). Acid β-glucosidase mutants linked to Gaucher disease, Parkinson disease, and Lewy body dementia alter α-synuclein processing. Annals of neurology *69*, 940-953.
Cummings, J.L. (1997). The Neuropsychiatric Inventory: assessing psychopathology in dementia patients. Neurology *48*, S10-16.
Cummings, J.L. (2004). Fluctuations in cognitive function in dementia with Lewy bodies. Lancet Neurol *3*, 266.
Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A., and Gornbein, J. (1994). The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology *44*, 2308-2314.
Curtain, C.C., Ali, F., Volitakis, I., Cherny, R.A., Norton, R.S., Beyreuther, K., Barrow, C.J., Masters, C.L., Bush, A.I., and Barnham, K.J. (2001). Alzheimer's disease amyloid-beta binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. The Journal of biological chemistry *276*, 20466-20473.
Davidsson, P., Puchades, M., and Blennow, K. (1999). Identification of synaptic vesicle, pre- and postsynaptic proteins in human cerebrospinal fluid using liquid-phase isoelectric focusing. Electrophoresis *20*, 431-437.
Davies, C.A., Mann, D.M., Sumpter, P.Q., and Yates, P.O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. J Neurol Sci *78*, 151-164.
De Deyn, P.P., Carrasco, M.M., Deberdt, W., Jeandel, C., Hay, D.P., Feldman, P.D., Young, C.A., Lehman, D.L., and Breier, A. (2004). Olanzapine versus placebo in the treatment of psychosis with or without associated behavioral disturbances in patients with Alzheimer's disease. Int J Geriatr Psychiatry *19*, 115-126.
de Medeiros, K., Robert, P., Gauthier, S., Stella, F., Politis, A., Leoutsakos, J., Taragano, F., Kremer, J., Brugnolo, A., Porsteinsson, A.P.*, et al.* (2010). The Neuropsychiatric Inventory-Clinician rating scale

(NPI-C): reliability and validity of a revised assessment of neuropsychiatric symptoms in dementia. Int Psychogeriatr *22*, 984-994.
DeCarli, C., Kawas, C., Morrison, J.H., Reuter-Lorenz, P.A., Sperling, R.A., and Wright, C.B. (2012). Session II: Mechanisms of age-related cognitive change and targets for intervention: neural circuits, networks, and plasticity. J Gerontol A Biol Sci Med Sci *67*, 747-753.
DeKosky, S.T., Scheff, S.W., and Styren, S.D. (1996). Structural correlates of cognition in dementia: quantification and assessment of synapse change. Neurodegeneration *5*, 417-421.
Delrieu, J., Ousset, P.J., Caillaud, C., and Vellas, B. (2012). 'Clinical trials in Alzheimer's disease': immunotherapy approaches. J Neurochem *120 Suppl 1*, 186-193.
Deshpande, A., Kawai, H., Metherate, R., Glabe, C.G., and Busciglio, J. (2009). A role for synaptic zinc in activity-dependent Abeta oligomer formation and accumulation at excitatory synapses. J Neurosci *29*, 4004-4015.
Desplats, P., Lee, H.J., Bae, E.J., Patrick, C., Rockenstein, E., Crews, L., Spencer, B., Masliah, E., and Lee, S.J. (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A *106*, 13010-13015.
Dexter, D.T., Wells, F.R., Lees, A.J., Agid, F., Agid, Y., Jenner, P., and Marsden, C.D. (1989). Increased nigral iron content and alterations in other metal ions occurring in brain in Parkinson's disease. Journal of neurochemistry *52*, 1830-1836.
Dickson, D.W. (2012). Parkinson's disease and parkinsonism: neuropathology. Cold Spring Harb Perspect Med *2*.
Dickson, D.W., Crystal, H.A., Mattiace, L.A., Masur, D.M., Blau, A.D., Davies, P., Yen, S.H., and Aronson, M.K. (1992). Identification of normal and pathological aging in prospectively studied nondemented elderly humans. Neurobiol Aging *13*, 179-189.
Dickson, D.W., Fujishiro, H., DelleDonne, A., Menke, J., Ahmed, Z., Klos, K.J., Josephs, K.A., Frigerio, R., Burnett, M., Parisi, J.E*., et al.* (2008). Evidence that incidental Lewy body disease is pre-symptomatic Parkinson's disease. Acta Neuropathol *115*, 437-444.
Drechsel, D.N., Hyman, A.A., Cobb, M.H., and Kirschner, M.W. (1992). Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol Biol Cell *3*, 1141-1154.
Duda, J.E. (2004). Pathology and neurotransmitter abnormalities of dementia with Lewy bodies. DementGeriatrCogn Disord *17 Suppl 1*, 3-14.
Duka, T., Rusnak, M., Drolet, R.E., Duka, V., Wersinger, C., Goudreau, J.L., and Sidhu, A. (2006). Alpha-synuclein induces hyperphosphorylation of Tau in the MPTP model of parkinsonism. FASEB J *20*, 2302-2312.
Duyckaerts, C., Delatour, B., and Potier, M.C. (2009). Classification and basic pathology of Alzheimer disease. Acta Neuropathol *118*, 5-36.
Duyckaerts, C., and Hauw, J.J. (1997). Prevalence, incidence and duration of Braak's stages in the general population: can we know? Neurobiol Aging *18*, 362-369; discussion 389-392.
Duyckaerts, C., Uchihara, T., Seilhean, D., He, Y., and Hauw, J.J. (1997). Dissociation of Alzheimer type pathology in a disconnected piece of cortex. Acta Neuropathol *93*, 501-507.
Eastwood, S.L., Heffernan, J., and Harrison, P.J. (1997). Chronic haloperidol treatment differentially affects the expression of synaptic and neuronal plasticity-associated genes. Mol Psychiatry *2*, 322-329.
Edison, P., Rowe, C.C., Rinne, J.O., Ng, S., Ahmed, I., Kemppainen, N., Villemagne, V.L., O'Keefe, G., Nagren, K., Chaudhury, K.R*., et al.* (2008). Amyloid load in Parkinson's disease dementia and Lewy body dementia measured with [11C]PIB positron emission tomography. J Neurol Neurosurg Psychiatry *79*, 1331-1338.
Edwards, D.R., Handsley, M.M., and Pennington, C.J. (2008). The ADAM metalloproteinases. Molecular aspects of medicine *29*, 258-289.
Ehrlich, I., and Malinow, R. (2004). Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience *24*, 916-927.

Ehrt, U., Broich, K., Larsen, J.P., Ballard, C., and Aarsland, D. (2010). Use of drugs with anticholinergic effect and impact on cognition in Parkinson's disease: a cohort study. J Neurol Neurosurg Psychiatry *81*, 160-165.
Emre, M., Aarsland, D., Albanese, A., Byrne, E.J., Deuschl, G., De Deyn, P.P., Durif, F., Kulisevsky, J., van Laar, T., Lees, A.*, et al.* (2004). Rivastigmine for dementia associated with Parkinson's disease. N Engl J Med *351*, 2509-2518.
Emre, M., Aarsland, D., Brown, R., Burn, D.J., Duyckaerts, C., Mizuno, Y., Broe, G.A., Cummings, J., Dickson, D.W., Gauthier, S.*, et al.* (2007). Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord *22*, 1689-1707; quiz 1837.
Eshkind, L.G., and Leube, R.E. (1995). Mice lacking synaptophysin reproduce and form typical synaptic vesicles. Cell Tissue Res *282*, 423-433.
Fasano, A., Daniele, A., and Albanese, A. (2012). Treatment of motor and non-motor features of Parkinson's disease with deep brain stimulation. Lancet Neurol *11*, 429-442.
Faux, N.G., Ritchie, C.W., Gunn, A., Rembach, A., Tsatsanis, A., Bedo, J., Harrison, J., Lannfelt, L., Blennow, K., Zetterberg, H.*, et al.* (2010). PBT2 rapidly improves cognition in Alzheimer's Disease: additional phase II analyses. J Alzheimers Dis *20*, 509-516.
Firbank, M.J., Colloby, S.J., Burn, D.J., McKeith, I.G., and O'Brien, J.T. (2003). Regional cerebral blood flow in Parkinson's disease with and without dementia. Neuroimage *20*, 1309-1319.
Fisher, A. (2012). Cholinergic modulation of amyloid precursor protein processing with emphasis on M1 muscarinic receptor: perspectives and challenges in treatment of Alzheimer's disease. J Neurochem *120 Suppl 1*, 22-33.
Folstein, M.F., Folstein, S.E., and McHugh, P.R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res *12*, 189-198.
Francis, P.T. (2009). Altered glutamate neurotransmission and behaviour in dementia: evidence from studies of memantine. Curr Mol Pharmacol *2*, 77-82.
Francis, P.T., Nordberg, A., and Arnold, S.E. (2005). A preclinical view of cholinesterase inhibitors in neuroprotection: do they provide more than symptomatic benefits in Alzheimer's disease? Trends Pharmacol Sci *26*, 104-111.
Francis, P.T., Palmer, A.M., Snape, M., and Wilcock, G.K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry *66*, 137-147.
Francis, P.T., Parsons, C.G., and Jones, R.W. (2012). Rationale for combining glutamatergic and cholinergic approaches in the symptomatic treatment of Alzheimer's disease. Expert Rev Neurother *12*, 1351-1365.
Frank, L., Kleinman, L., Ciesla, G., Rupnow, M.F., and Brodaty, H. (2004). The effect of risperidone on nursing burden associated with caring for patients with dementia. J Am Geriatr Soc *52*, 1449-1455.
Frederickson, C.J., Suh, S.W., Silva, D., and Thompson, R.B. (2000). Importance of zinc in the central nervous system: the zinc-containing neuron. J Nutr *130*, 1471S-1483S.
Fritze, F., Ehrt, U., Hortobagyi, T., Ballard, C., and Aarsland, D. (2011a). Depressive symptoms in Alzheimer's disease and lewy body dementia: a one-year follow-up study. Dement Geriatr Cogn Disord *32*, 143-149.
Fritze, F., Ehrt, U., Sønnesyn, H., Kurz, M., Hortobágyi, T., Nore, S.P., Ballard, C., and Aarsland, D. (2011b). Depression in mild dementia: associations with diagnosis, APOE genotype and clinical features. Int J Geriatr Psychiatry *26*, 1054-1061.
Fuchs, J., Nilsson, C., Kachergus, J., Munz, M., Larsson, E.M., Schüle, B., Langston, J.W., Middleton, F.A., Ross, O.A., Hulihan, M.*, et al.* (2007). Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. Neurology *68*, 916-922.
Fuster, J.M. (2001). The prefrontal cortex--an update: time is of the essence. Neuron *30*, 319-333.
Gabriel, S.M., Haroutunian, V., Powchik, P., Honer, W.G., Davidson, M., Davies, P., and Davis, K.L. (1997). Increased concentrations of presynaptic proteins in the cingulate cortex of subjects with schizophrenia. Arch Gen Psychiatry *54*, 559-566.

Gaither, L.A., and Eide, D.J. (2001). Eukaryotic zinc transporters and their regulation. Biometals *14*, 251-270.
Ganguli, M., Ratcliff, G., Huff, F.J., Belle, S., Kancel, M.J., Fischer, L., and Kuller, L.H. (1990). Serial sevens versus world backwards: a comparison of the two measures of attention from the MMSE. J Geriatr Psychiatry Neurol *3*, 203-207.
Garcia-Reitbock, P., Anichtchik, O., Bellucci, A., Iovino, M., Ballini, C., Fineberg, E., Ghetti, B., Della Corte, L., Spano, P., Tofaris, G.K.*, et al.* (2010). SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. Brain *133*, 2032-2044.
García-Colunga, J., Reyes-Haro, D., Godoy-García, I.U., and Miledi, R. (2005). Zinc modulation of serotonin uptake in the adult rat corpus callosum. J Neurosci Res *80*, 145-149.
Garringer, H.J., Murrell, J., Sammeta, N., Gnezda, A., Ghetti, B., and Vidal, R. (2013). Increased tau phosphorylation and tau truncation, and decreased synaptophysin levels in mutant BRI2/tau transgenic mice. PLoS One *8*, e56426.
Gauthier, S., Cummings, J., Ballard, C., Brodaty, H., Grossberg, G., Robert, P., and Lyketsos, C. (2010). Management of behavioral problems in Alzheimer's disease. Int Psychogeriatr *22*, 346-372.
Geerts, H., and Grossberg, G.T. (2006). Pharmacology of acetylcholinesterase inhibitors and N-methyl-D-aspartate receptors for combination therapy in the treatment of Alzheimer's disease. J Clin Pharmacol *46*, 8S-16S.
Glenner, G.G., and Wong, C.W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun *120*, 885-890.
Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., and James, L. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature *349*, 704-706.
Goate, A., and Hardy, J. (2012). Twenty years of Alzheimer's disease-causing mutations. J Neurochem *120 Suppl 1*, 3-8.
Goedert, M. (2001). Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci *2*, 492-501.
Goker-Alpan, O., Giasson, B.I., Eblan, M.J., Nguyen, J., Hurtig, H.I., Lee, V.M., Trojanowski, J.Q., and Sidransky, E. (2006). Glucocerebrosidase mutations are an important risk factor for Lewy body disorders. Neurology *67*, 908-910.
Golts, N., Snyder, H., Frasier, M., Theisler, C., Choi, P., and Wolozin, B. (2002). Magnesium inhibits spontaneous and iron-induced aggregation of alpha-synuclein. The Journal of biological chemistry *277*, 16116-16123.
Gomperts, S.N., Rentz, D.M., Moran, E., Becker, J.A., Locascio, J.J., Klunk, W.E., Mathis, C.A., Elmaleh, D.R., Shoup, T., Fischman, A.J.*, et al.* (2008). Imaging amyloid deposition in Lewy body diseases. Neurology *71*, 903-910.
González, C., Martín, T., Cacho, J., Breñas, M.T., Arroyo, T., García-Berrocal, B., Navajo, J.A., and González-Buitrago, J.M. (1999). Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. Eur J Clin Invest *29*, 637-642.
Gorell, J.M., Johnson, C.C., Rybicki, B.A., Peterson, E.L., Kortsha, G.X., Brown, G.G., and Richardson, R.J. (1999). Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. Neurotoxicology *20*, 239-247.
Greffard, S., Verny, M., Bonnet, A.M., Seilhean, D., Hauw, J.J., and Duyckaerts, C. (2010). A stable proportion of Lewy body bearing neurons in the substantia nigra suggests a model in which the Lewy body causes neuronal death. Neurobiol Aging *31*, 99-103.
Guerreiro, R.J., and Hardy, J. (2011). Alzheimer's disease genetics: lessons to improve disease modelling. Biochem Soc Trans *39*, 910-916.
Gundelfinger, E.D., Boeckers, T.M., Baron, M.K., and Bowie, J.U. (2006). A role for zinc in postsynaptic density asSAMbly and plasticity? Trends Biochem Sci *31*, 366-373.

Guzowski, J.F. (2002). Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus *12*, 86-104.
Gylys, K.H., Fein, J.A., Yang, F., Wiley, D.J., Miller, C.A., and Cole, G.M. (2004). Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence. Am J Pathol *165*, 1809-1817.
Gómez-Isla, T., Growdon, W.B., McNamara, M., Newell, K., Gómez-Tortosa, E., Hedley-Whyte, E.T., and Hyman, B.T. (1999). Clinicopathologic correlates in temporal cortex in dementia with Lewy bodies. Neurology *53*, 2003-2009.
Güntert, A., Döbeli, H., and Bohrmann, B. (2006). High sensitivity analysis of amyloid-beta peptide composition in amyloid deposits from human and PS2APP mouse brain. Neuroscience *143*, 461-475.
Hall, K.A., and O'Connor, D.W. (2004). Correlates of aggressive behavior in dementia. Int Psychogeriatr *16*, 141-158.
Hamilton, R.L. (2000). Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. Brain Pathol *10*, 378-384.
Han, K., and Kim, E. (2008). Synaptic adhesion molecules and PSD-95. ProgNeurobiol *84*, 263-283.
Hansen, C., and Li, J.Y. (2012). Beyond alpha-synuclein transfer: pathology propagation in Parkinson's disease. Trends Mol Med *18*, 248-255.
Hansen, L., Salmon, D., Galasko, D., Masliah, E., Katzman, R., DeTeresa, R., Thal, L., Pay, M.M., Hofstetter, R., and Klauber, M. (1990). The Lewy body variant of Alzheimer's disease: a clinical and pathologic entity. Neurology *40*, 1-8.
Hansen, L.A., Daniel, S.E., Wilcock, G.K., and Love, S. (1998). Frontal cortical synaptophysin in Lewy body diseases: relation to Alzheimer's disease and dementia. J Neurol Neurosurg Psychiatry *64*, 653-656.
Harding, A.J., Broe, G.A., and Halliday, G.M. (2002). Visual hallucinations in Lewy body disease relate to Lewy bodies in the temporal lobe. Brain *125*, 391-403.
Harding, A.J., and Halliday, G.M. (2001). Cortical Lewy body pathology in the diagnosis of dementia. Acta Neuropathol *102*, 355-363.
Hardy, J. (2009). The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem *110*, 1129-1134.
Hardy, J., and Allsop, D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci *12*, 383-388.
Hardy, J., Lewis, P., Revesz, T., Lees, A., and Paisan-Ruiz, C. (2009). The genetics of Parkinson's syndromes: a critical review. Curr Opin Genet Dev *19*, 254-265.
Hawkes, C.H., Del Tredici, K., and Braak, H. (2009). Parkinson's disease: the dual hit theory revisited. Ann N Y Acad Sci *1170*, 615-622.
Hely, M.A., Reid, W.G., Adena, M.A., Halliday, G.M., and Morris, J.G. (2008). The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Mov Disord *23*, 837-844.
Hering, H., and Sheng, M. (2001). Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci *2*, 880-888.
Herrmann, N., Li, A., and Lanctot, K. (2011). Memantine in dementia: a review of the current evidence. Expert Opin Pharmacother *12*, 787-800.
Hirzel, K., Müller, U., Latal, A.T., Hülsmann, S., Grudzinska, J., Seeliger, M.W., Betz, H., and Laube, B. (2006). Hyperekplexia phenotype of glycine receptor alpha1 subunit mutant mice identifies Zn(2+) as an essential endogenous modulator of glycinergic neurotransmission. Neuron *52*, 679-690.
Ho, A., and Shen, J. (2011). Presenilins in synaptic function and disease. Trends Mol Med *17*, 617-624.
Hollenbeck, P.J. (2005). Mitochondria and neurotransmission: evacuating the synapse. Neuron *47*, 331-333.

Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., Jones, R.W., Bullock, R., Love, S., Neal, J.W*., et al.* (2008). Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet *372*, 216-223.
Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B.I., Geschwind, D.H., Bird, T.D., McKeel, D., Goate, A*., et al.* (1998). Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. Science *282*, 1914-1917.
Honoré, T., Lauridsen, J., and Krogsgaard-Larsen, P. (1982). The binding of [3H]AMPA, a structural analogue of glutamic acid, to rat brain membranes. J Neurochem *38*, 173-178.
Howard, R., McShane, R., Lindesay, J., Ritchie, C., Baldwin, A., Barber, R., Burns, A., Dening, T., Findlay, D., Holmes, C*., et al.* (2012). Donepezil and memantine for moderate-to-severe Alzheimer's disease. N Engl J Med *366*, 893-903.
Howlett, D.R., Hortobágyi, T., and Francis, P.T. (2013). Clusterin Associates Specifically with Aβ40 in Alzheimer's Disease Brain Tissue. Brain Pathol.
Huang, L., and Tepaamorndech, S. (2013). The SLC30 family of zinc transporters - A review of current understanding of their biological and pathophysiological roles. Mol Aspects Med *34*, 548-560.
Hung, Y.H., Robb, E.L., Volitakis, I., Ho, M., Evin, G., Li, Q.X., Culvenor, J.G., Masters, C.L., Cherny, R.A., and Bush, A.I. (2009). Paradoxical condensation of copper with elevated beta-amyloid in lipid rafts under cellular copper deficiency conditions: implications for Alzheimer disease. The Journal of biological chemistry *284*, 21899-21907.
Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A*., et al.* (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature *393*, 702-705.
Hwang, D.Y., Jee, S.W., Lee, S.H., Bae, C.J., Goo, J.S., Kim, J.E., Nam, S.H., Choi, S.I., Lee, H.R., Shim, S.B*., et al.* (2011). Aβ-42 deposition significantly increases the insolubility of synaptophysin in the brains of hAPPsw/hPS2m double transgenic mice. Int J Mol Med *28*, 223-229.
Héraud, C., Goufak, D., Ando, K., Leroy, K., Suain, V., Yilmaz, Z., De Decker, R., Authelet, M., Laporte, V., Octave, J.N*., et al.* (2013). Increased misfolding and truncation of tau in APP/PS1/tau transgenic mice compared to mutant tau mice. Neurobiol Dis.
Ikeda, K., Haga, C., Oyanagi, S., Iritani, S., and Kosaka, K. (1992). Ultrastructural and immunohistochemical study of degenerate neurite-bearing ghost tangles. J Neurol *239*, 191-194.
Imamura, T., Ishii, K., Sasaki, M., Kitagaki, H., Yamaji, S., Hirono, N., Shimomura, T., Hashimoto, M., Tanimukai, S., Kazui, H*., et al.* (1997). Regional cerebral glucose metabolism in dementia with Lewy bodies and Alzheimer's disease: a comparative study using positron emission tomography. Neurosci Lett *235*, 49-52.
Ishibashi, K., Tomiyama, T., Nishitsuji, K., Hara, M., and Mori, H. (2006). Absence of synaptophysin near cortical neurons containing oligomer Abeta in Alzheimer's disease brain. J Neurosci Res *84*, 632-636.
Ishii, K., Yamaji, S., Kitagaki, H., Imamura, T., Hirono, N., and Mori, E. (1999). Regional cerebral blood flow difference between dementia with Lewy bodies and AD. Neurology *53*, 413-416.
Ittner, L.M., and Gotz, J. (2011). Amyloid-beta and tau--a toxic pas de deux in Alzheimer's disease. Nat Rev Neurosci *12*, 65-72.
Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wölfing, H., Chieng, B.C., Christie, M.J., Napier, I.A*., et al.* (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell *142*, 387-397.
Ivanov, A., Esclapez, M., and Ferhat, L. (2009). Role of drebrin A in dendritic spine plasticity and synaptic function: Implications in neurological disorders. CommunIntegrBiol *2*, 268-270.
Jack, C.R., Jr., Lowe, V.J., Senjem, M.L., Weigand, S.D., Kemp, B.J., Shiung, M.M., Knopman, D.S., Boeve, B.F., Klunk, W.E., Mathis, C.A*., et al.* (2008). 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. Brain *131*, 665-680.

Jahn, R., and Fasshauer, D. (2012). Molecular machines governing exocytosis of synaptic vesicles. Nature *490*, 201-207.
Jakes, R., Spillantini, M.G., and Goedert, M. (1994). Identification of two distinct synucleins from human brain. FEBS Lett *345*, 27-32.
Jankovic, J., McDermott, M., Carter, J., Gauthier, S., Goetz, C., Golbe, L., Huber, S., Koller, W., Olanow, C., and Shoulson, I. (1990). Variable expression of Parkinson's disease: a base-line analysis of the DATATOP cohort. The Parkinson Study Group. Neurology *40*, 1529-1534.
Jellinger, K.A. (2004). Lewy body-related alpha-synucleinopathy in the aged human brain. J Neural Transm *111*, 1219-1235.
Jellinger, K.A. (2008). A critical reappraisal of current staging of Lewy-related pathology in human brain. Acta Neuropathol *116*, 1-16.
Jellinger, K.A. (2011). Interaction between α-synuclein and tau in Parkinson's disease comment on Wills et al.: elevated tauopathy and α-synuclein pathology in postmortem Parkinson's disease brains with and without dementia. Exp Neurol 2010; 225: 210-218. Exp Neurol *227*, 13-18.
Jellinger, K.A., and Attems, J. (2008). Prevalence and impact of vascular and Alzheimer pathologies in Lewy body disease. Acta Neuropathol *115*, 427-436.
Jellinger, K.A., and Attems, J. (2012). Neuropathology and general autopsy findings in nondemented aged subjects. Clin Neuropathol *31*, 87-98.
Jensen, P.H., Hager, H., Nielsen, M.S., Hojrup, P., Gliemann, J., and Jakes, R. (1999). alpha-synuclein binds to Tau and stimulates the protein kinase A-catalyzed tau phosphorylation of serine residues 262 and 356. J Biol Chem *274*, 25481-25489.
Johnston, N.L., Cervenak, J., Shore, A.D., Torrey, E.F., Yolken, R.H., and Cerevnak, J. (1997). Multivariate analysis of RNA levels from postmortem human brains as measured by three different methods of RT-PCR. Stanley Neuropathology Consortium. J Neurosci Methods *77*, 83-92.
Jomova, K., Vondrakova, D., Lawson, M., and Valko, M. (2010). Metals, oxidative stress and neurodegenerative disorders. Molecular and cellular biochemistry *345*, 91-104.
Joshi, M., Akhtar, M., Najmi, A.K., Khuroo, A.H., and Goswami, D. (2012). Effect of zinc in animal models of anxiety, depression and psychosis. Hum Exp Toxicol *31*, 1237-1243.
Jucker, M., and Walker, L.C. (2011). Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. Ann Neurol *70*, 532-540.
Kaiser, J. (1994). Alzheimer's: could there be a zinc link? Science *265*, 1365.
Kalaitzakis, M.E., Graeber, M.B., Gentleman, S.M., and Pearce, R.K. (2008). The dorsal motor nucleus of the vagus is not an obligatory trigger site of Parkinson's disease: a critical analysis of alpha-synuclein staging. Neuropathol Appl Neurobiol *34*, 284-295.
Kane, M.D., Lipinski, W.J., Callahan, M.J., Bian, F., Durham, R.A., Schwarz, R.D., Roher, A.E., and Walker, L.C. (2000). Evidence for seeding of beta -amyloid by intracerebral infusion of Alzheimer brain extracts in beta -amyloid precursor protein-transgenic mice. J Neurosci *20*, 3606-3611.
Kantheti, P., Qiao, X., Diaz, M.E., Peden, A.A., Meyer, G.E., Carskadon, S.L., Kapfhamer, D., Sufalko, D., Robinson, M.S., Noebels, J.L*., et al.* (1998). Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. Neuron *21*, 111-122.
Kastner, S., Pinsk, M.A., De Weerd, P., Desimone, R., and Ungerleider, L.G. (1999). Increased activity in human visual cortex during directed attention in the absence of visual stimulation. Neuron *22*, 751-761.
Katzenschlager, R., Sampaio, C., Costa, J., and Lees, A. (2003). Anticholinergics for symptomatic management of Parkinson's disease. Cochrane Database Syst Rev, CD003735.
Kelly, S.J., Ostrowski, N.L., and Wilson, M.A. (1999). Gender differences in brain and behavior: hormonal and neural bases. Pharmacol Biochem Behav *64*, 655-664.
Kenny, E.R., Blamire, A.M., Firbank, M.J., and O'Brien, J.T. (2012). Functional connectivity in cortical regions in dementia with Lewy bodies and Alzheimer's disease. Brain *135*, 569-581.
Kester, M.I., and Scheltens, P. (2009). Dementia: the bare essentials. PractNeurol *9*, 241-251.

Kim, E., and Sheng, M. (2004). PDZ domain proteins of synapses. Nature reviews Neuroscience *5*, 771-781.
Kim, T.D., Paik, S.R., Yang, C.H., and Kim, J. (2000). Structural changes in alpha-synuclein affect its chaperone-like activity in vitro. Protein science : a publication of the Protein Society *9*, 2489-2496.
Kirvell, S.L., Esiri, M., and Francis, P.T. (2006). Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. J Neurochem *98*, 939-950.
Klatka, L.A., Louis, E.D., and Schiffer, R.B. (1996). Psychiatric features in diffuse Lewy body disease: a clinicopathologic study using Alzheimer's disease and Parkinson's disease comparison groups. Neurology *47*, 1148-1152.
Klucken, J., Ingelsson, M., Shin, Y., Irizarry, M.C., Hedley-Whyte, E.T., Frosch, M., Growdon, J., McLean, P., and Hyman, B.T. (2006). Clinical and biochemical correlates of insoluble alpha-synuclein in dementia with Lewy bodies. Acta Neuropathol *111*, 101-108.
Klucken, J., McLean, P.J., Gomez-Tortosa, E., Ingelsson, M., and Hyman, B.T. (2003). Neuritic alterations and neural system dysfunction in Alzheimer's disease and dementia with Lewy bodies. Neurochem Res *28*, 1683-1691.
Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D.P., Bergstrom, M., Savitcheva, I., Huang, G.F., Estrada, S*., et al.* (2004). Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol *55*, 306-319.
Klyubin, I., Walsh, D.M., Lemere, C.A., Cullen, W.K., Shankar, G.M., Betts, V., Spooner, E.T., Jiang, L., Anwyl, R., Selkoe, D.J*., et al.* (2005). Amyloid beta protein immunotherapy neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. Nat Med *11*, 556-561.
Knopman, D.S., Parisi, J.E., Salviati, A., Floriach-Robert, M., Boeve, B.F., Ivnik, R.J., Smith, G.E., Dickson, D.W., Johnson, K.A., Petersen, L.E*., et al.* (2003). Neuropathology of cognitively normal elderly. J Neuropathol Exp Neurol *62*, 1087-1095.
Knott, A.B., Perkins, G., Schwarzenbacher, R., and Bossy-Wetzel, E. (2008). Mitochondrial fragmentation in neurodegeneration. Nat Rev Neurosci *9*, 505-518.
Kosik, K.S., Joachim, C.L., and Selkoe, D.J. (1986). Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A *83*, 4044-4048.
Kramer, M.L., and Schulz-Schaeffer, W.J. (2007). Presynaptic {alpha}-Synuclein Aggregates, Not Lewy Bodies, Cause Neurodegeneration in Dementia with Lewy Bodies. Journal of Neuroscience *27*, 1405-1410.
Krishnan, K.R., Charles, H.C., Doraiswamy, P.M., Mintzer, J., Weisler, R., Yu, X., Perdomo, C., Ieni, J.R., and Rogers, S. (2003). Randomized, placebo-controlled trial of the effects of donepezil on neuronal markers and hippocampal volumes in Alzheimer's disease. Am J Psychiatry *160*, 2003-2011.
Kruger, N.J. (1994). The Bradford method for protein quantitation. Methods Mol Biol *32*, 9-15.
Kuhn, M., Haebig, K., Bonin, M., Ninkina, N., Buchman, V.L., Poths, S., and Riess, O. (2007). Whole genome expression analyses of single- and double-knock-out mice implicate partially overlapping functions of alpha- and gamma-synuclein. Neurogenetics *8*, 71-81.
Kukull, W.A., Larson, E.B., Teri, L., Bowen, J., McCormick, W., and Pfanschmidt, M.L. (1994). The Mini-Mental State Examination score and the clinical diagnosis of dementia. J Clin Epidemiol *47*, 1061-1067.
Kuret, J., Congdon, E.E., Li, G., Yin, H., Yu, X., and Zhong, Q. (2005). Evaluating triggers and enhancers of tau fibrillization. Microsc Res Tech *67*, 141-155.
Kurz, M.W., Schlitter, A.M., Larsen, J.P., Ballard, C., and Aarsland, D. (2006). Familial occurrence of dementia and parkinsonism: a systematic review. Dementia and geriatric cognitive disorders *22*, 288-295.
Kövari, E., Gold, G., Herrmann, F.R., Canuto, A., Hof, P.R., Bouras, C., and Giannakopoulos, P. (2003). Lewy body densities in the entorhinal and anterior cingulate cortex predict cognitive deficits in Parkinson's disease. Acta Neuropathol *106*, 83-88.

Lacor, P.N., Buniel, M.C., Chang, L., Fernandez, S.J., Gong, Y., Viola, K.L., Lambert, M.P., Velasco, P.T., Bigio, E.H., Finch, C.E.*, et al.* (2004). Synaptic targeting by Alzheimer's-related amyloid beta oligomers. The Journal of neuroscience : the official journal of the Society for Neuroscience *24*, 10191-10200.
Lahiri, D.K., and Maloney, B. (2010). Beyond the signaling effect role of amyloid-ss42 on the processing of APP, and its clinical implications. Exp Neurol *225*, 51-54.
Lahr, D., Beblo, T., and Hartje, W. (2007). Cognitive performance and subjective complaints before and after remission of major depression. Cogn Neuropsychiatry *12*, 25-45.
Lannfelt, L., Blennow, K., Zetterberg, H., Batsman, S., Ames, D., Harrison, J., Masters, C.L., Targum, S., Bush, A.I., Murdoch, R.*, et al.* (2008). Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. Lancet Neurol *7*, 779-786.
Lantos, P.L., Ovenstone, I.M., Johnson, J., Clelland, C.A., Roques, P., and Rossor, M.N. (1994). Lewy bodies in the brain of two members of a family with the 717 (Val to Ile) mutation of the amyloid precursor protein gene. Neurosci Lett *172*, 77-79.
Lasagna-Reeves, C.A., Castillo-Carranza, D.L., Sengupta, U., Clos, A.L., Jackson, G.R., and Kayed, R. (2011). Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. Mol Neurodegener *6*, 39.
Lee, G., Neve, R.L., and Kosik, K.S. (1989). The microtubule binding domain of tau protein. Neuron *2*, 1615-1624.
Lee, J.Y., Cho, E., Kim, T.Y., Kim, D.K., Palmiter, R.D., Volitakis, I., Kim, J.S., Bush, A.I., and Koh, J.Y. (2010). Apolipoprotein E ablation decreases synaptic vesicular zinc in the brain. Biometals *23*, 1085-1095.
Lee, J.Y., Cole, T.B., Palmiter, R.D., Suh, S.W., and Koh, J.Y. (2002). Contribution by synaptic zinc to the gender-disparate plaque formation in human Swedish mutant APP transgenic mice. Proc Natl Acad Sci U S A *99*, 7705-7710.
Lee, M.J., Lee, J.H., and Rubinsztein, D.C. (2013). Tau degradation: the ubiquitin-proteasome system versus the autophagy-lysosome system. Prog Neurobiol *105*, 49-59.
Leng, Y., Chase, T.N., and Bennett, M.C. (2001). Muscarinic receptor stimulation induces translocation of an alpha-synuclein oligomer from plasma membrane to a light vesicle fraction in cytoplasm. J Biol Chem *276*, 28212-28218.
Lesné, S., Koh, M.T., Kotilinek, L., Kayed, R., Glabe, C.G., Yang, A., Gallagher, M., and Ashe, K.H. (2006). A specific amyloid-beta protein assembly in the brain impairs memory. Nature *440*, 352-357.
Leuba, G., Savioz, A., Vernay, A., Carnal, B., Kraftsik, R., Tardif, E., Riederer, I., and Riederer, B.M. (2008). Differential changes in synaptic proteins in the Alzheimer frontal cortex with marked increase in PSD-95 postsynaptic protein. JAlzheimersDis *15*, 139-151.
Leung, K.W., Liu, M., Xu, X., Seiler, M.J., Barnstable, C.J., and Tombran-Tink, J. (2008). Expression of ZnT and ZIP zinc transporters in the human RPE and their regulation by neurotrophic factors. Invest Ophthalmol Vis Sci *49*, 1221-1231.
Levenson, C.W. (2006). Zinc: the new antidepressant? Nutr Rev *64*, 39-42.
Leverenz, J.B., Umar, I., Wang, Q., Montine, T.J., McMillan, P.J., Tsuang, D.W., Jin, J., Pan, C., Shin, J., Zhu, D.*, et al.* (2007). Proteomic identification of novel proteins in cortical lewy bodies. Brain Pathol *17*, 139-145.
Li, L., Sengupta, A., Haque, N., Grundke-Iqbal, I., and Iqbal, K. (2004). Memantine inhibits and reverses the Alzheimer type abnormal hyperphosphorylation of tau and associated neurodegeneration. FEBS Lett *566*, 261-269.
Lindenmayer, J.P. (2000). The pathophysiology of agitation. J Clin Psychiatry *61 Suppl 14*, 5-10.
Linkous, D.H., Flinn, J.M., Koh, J.Y., Lanzirotti, A., Bertsch, P.M., Jones, B.F., Giblin, L.J., and Frederickson, C.J. (2008). Evidence that the ZNT3 protein controls the total amount of elemental zinc in synaptic vesicles. JHistochemCytochem *56*, 3-6.

Lippa, C.F., Smith, T.W., and Swearer, J.M. (1994). Alzheimer's disease and Lewy body disease: a comparative clinicopathological study. Ann Neurol *35*, 81-88.
Liu, G., Aliaga, L., and Cai, H. (2012). α-synuclein, LRRK2 and their interplay in Parkinson's disease. Future Neurol *7*, 145-153.
Love, S., Siew, L.K., Dawbarn, D., Wilcock, G.K., Ben-Shlomo, Y., and Allen, S.J. (2006). Premorbid effects of APOE on synaptic proteins in human temporal neocortex. Neurobiol Aging *27*, 797-803.
Lovell, M.A., Robertson, J.D., Teesdale, W.J., Campbell, J.L., and Markesbery, W.R. (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. Journal of the neurological sciences *158*, 47-52.
Lundstrom, B.N., Ingvar, M., and Petersson, K.M. (2005). The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. Neuroimage *27*, 824-834.
Maes, M., Vandoolaeghe, E., Neels, H., Demedts, P., Wauters, A., Meltzer, H.Y., Altamura, C., and Desnyder, R. (1997). Lower serum zinc in major depression is a sensitive marker of treatment resistance and of the immune/inflammatory response in that illness. Biol Psychiatry *42*, 349-358.
Majdi, M., Ribeiro-da-Silva, A., and Cuello, A.C. (2007). Cognitive impairment and transmitter-specific pre- and postsynaptic changes in the rat cerebral cortex during ageing. Eur J Neurosci *26*, 3583-3596.
Marchesi, V.T. (2012). Alzheimer's disease 2012: the great amyloid gamble. Am J Pathol *180*, 1762-1767.
Marra, C., Quaranta, D., Profice, P., Pilato, F., Capone, F., Iodice, F., Di Lazzaro, V., and Gainotti, G. (2012). Central cholinergic dysfunction measured "in vivo" correlates with different behavioral disorders in Alzheimer's disease and dementia with Lewy body. Brain Stimul *5*, 533-538.
Marui, W., Iseki, E., Nakai, T., Miura, S., Kato, M., Uéda, K., and Kosaka, K. (2002). Progression and staging of Lewy pathology in brains from patients with dementia with Lewy bodies. J Neurol Sci *195*, 153-159.
Masliah, E., Mallory, M., Alford, M., DeTeresa, R., Hansen, L.A., McKeel, D.W., and Morris, J.C. (2001a). Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology *56*, 127-129.
Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M., and Mucke, L. (2001b). beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci U S A *98*, 12245-12250.
Masliah, E., Terry, R.D., DeTeresa, R.M., and Hansen, L.A. (1989). Immunohistochemical quantification of the synapse-related protein synaptophysin in Alzheimer disease. Neurosci Lett *103*, 234-239.
Mattila, P.M., Rinne, J.O., Helenius, H., Dickson, D.W., and Röyttä, M. (2000). Alpha-synuclein-immunoreactive cortical Lewy bodies are associated with cognitive impairment in Parkinson's disease. Acta Neuropathol *100*, 285-290.
McKeith, I. (2009). Commentary: DLB and PDD: the same or different? Is there a debate? Int Psychogeriatr *21*, 220-224.
McKeith, I., Fairbairn, A., Perry, R., Thompson, P., and Perry, E. (1992). Neuroleptic sensitivity in patients with senile dementia of Lewy body type. BMJ *305*, 673-678.
McKeith, I., Mintzer, J., Aarsland, D., Burn, D., Chiu, H., Cohen-Mansfield, J., Dickson, D., Dubois, B., Duda, J.E., Feldman, H.*, et al.* (2004). Dementia with Lewy bodies. The Lancet Neurology *3*, 19-28.
McKeith, I.G., Dickson, D.W., Lowe, J., Emre, M., O'Brien, J.T., Feldman, H., Cummings, J., Duda, J.E., Lippa, C., Perry, E.K.*, et al.* (2005). Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology *65*, 1863-1872.
McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack, C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R.*, et al.* (2011). The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement *7*, 263-269.
McLoughlin, I.J., and Hodge, J.S. (1990). Zinc in depressive disorder. Acta Psychiatr Scand *82*, 451-453.

McMahon, H.T., Bolshakov, V.Y., Janz, R., Hammer, R.E., Siegelbaum, S.A., and Sudhof, T.C. (1996). Synaptophysin, a major synaptic vesicle protein, is not essential for neurotransmitter release. Proc Natl Acad Sci U S A *93*, 4760-4764.
McShane, R., Areosa Sastre, A., and Minakaran, N. (2006). Memantine for dementia. Cochrane Database Syst Rev, CD003154.
McShane, R., Keene, J., Gedling, K., Fairburn, C., Jacoby, R., and Hope, T. (1997). Do neuroleptic drugs hasten cognitive decline in dementia? Prospective study with necropsy follow up. BMJ *314*, 266-270.
Medina, D.A., and Gaviria, M. (2008). Diffusion tensor imaging investigations in Alzheimer's disease: the resurgence of white matter compromise in the cortical dysfunction of the aging brain. Neuropsychiatr Dis Treat *4*, 737-742.
Meeus, B., Theuns, J., and Van Broeckhoven, C. (2012). The Genetics of Dementia With Lewy Bodies: What Are We Missing?Genetics of Dementia With Lewy Bodies. Arch Neurol, 1-6.
Middelkoop, H.A., van der Flier, W.M., Burton, E.J., Lloyd, A.J., Paling, S., Barber, R., Ballard, C., McKeith, I.G., and O'Brien, J.T. (2001). Dementia with Lewy bodies and AD are not associated with occipital lobe atrophy on MRI. Neurology *57*, 2117-2120.
Migaud, M., Charlesworth, P., Dempster, M., Webster, L.C., Watabe, A.M., Makhinson, M., He, Y., Ramsay, M.F., Morris, R.G., Morrison, J.H.*, et al.* (1998). Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. Nature *396*, 433-439.
Milner, B. (2003). Visual recognition and recall after right temporal-lobe excision in man. Epilepsy Behav *4*, 799-812.
Minger, S.L., Honer, W.G., Esiri, M.M., McDonald, B., Keene, J., Nicoll, J.A., Carter, J., Hope, T., and Francis, P.T. (2001). Synaptic pathology in prefrontal cortex is present only with severe dementia in Alzheimer disease. J Neuropathol Exp Neurol *60*, 929-936.
Mirra, S.S., Heyman, A., McKeel, D., Sumi, S.M., Crain, B.J., Brownlee, L.M., Vogel, F.S., Hughes, J.P., van Belle, G., and Berg, L. (1991). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology *41*, 479-486.
Mo, Z.Y., Zhu, Y.Z., Zhu, H.L., Fan, J.B., Chen, J., and Liang, Y. (2009). Low micromolar zinc accelerates the fibrillization of human tau via bridging of Cys-291 and Cys-322. J Biol Chem *284*, 34648-34657.
Moh, C.F., Siedlak, S.L., Tabaton, M., Perry, G., Castellani, R.J., and Smith, M.A. (2010). Paraffin-embedded tissue (PET) blot method: application to Alzheimer disease. J Neurosci Methods *190*, 244-247.
Monsch, A.U., Foldi, N.S., Ermini-Fünfschilling, D.E., Berres, M., Taylor, K.I., Seifritz, E., Stähelin, H.B., and Spiegel, R. (1995). Improving the diagnostic accuracy of the Mini-Mental State Examination. Acta Neurol Scand *92*, 145-150.
Moraes, C.F., Lins, T.C., Carmargos, E.F., Naves, J.O., Pereira, R.W., and Nobrega, O.T. (2012). Lessons from genome-wide association studies findings in Alzheimer's disease. Psychogeriatrics *12*, 62-73.
Mori, E., Hashimoto, M., Krishnan, K.R., and Doraiswamy, P.M. (2006). What constitutes clinical evidence for neuroprotection in Alzheimer disease: support for the cholinesterase inhibitors? Alzheimer Dis Assoc Disord *20*, S19-26.
Morris, J.C., Aisen, P.S., Bateman, R.J., Benzinger, T.L., Cairns, N.J., Fagan, A.M., Ghetti, B., Goate, A.M., Holtzman, D.M., Klunk, W.E.*, et al.* (2012). Developing an international network for Alzheimer research: The Dominantly Inherited Alzheimer Network. Clin Investig (Lond) *2*, 975-984.
Mufson, E.J., Binder, L., Counts, S.E., DeKosky, S.T., de Toledo-Morrell, L., Ginsberg, S.D., Ikonomovic, M.D., Perez, S.E., and Scheff, S.W. (2012). Mild cognitive impairment: pathology and mechanisms. Acta Neuropathol *123*, 13-30.
Murray, A.D. (2011). Imaging Approaches for Dementia. AJNR Am J Neuroradiol.
Müller, B.M., Kistner, U., Kindler, S., Chung, W.J., Kuhlendahl, S., Fenster, S.D., Lau, L.F., Veh, R.W., Huganir, R.L., Gundelfinger, E.D.*, et al.* (1996). SAP102, a novel postsynaptic protein that interacts with NMDA receptor complexes in vivo. Neuron *17*, 255-265.

Müller, N.G., and Knight, R.T. (2006). The functional neuroanatomy of working memory: contributions of human brain lesion studies. Neuroscience *139*, 51-58.
Młyniec, K., Davies, C.L., Budziszewska, B., Opoka, W., Reczyński, W., Sowa-Kućma, M., Doboszewska, U., Pilc, A., and Nowak, G. (2012). Time course of zinc deprivation-induced alterations of mice behavior in the forced swim test. Pharmacol Rep *64*, 567-575.
Nagahama, Y., Okina, T., Suzuki, N., and Matsuda, M. (2010). Neural correlates of psychotic symptoms in dementia with Lewy bodies. Brain *133*, 557-567.
Nagano-Saito, A., Washimi, Y., Arahata, Y., Iwai, K., Kawatsu, S., Ito, K., Nakamura, A., Abe, Y., Yamada, T., Kato, T.*, et al.* (2004). Visual hallucination in Parkinson's disease with FDG PET. Mov Disord *19*, 801-806.
Nakano, S., Asada, T., Matsuda, H., Uno, M., and Takasaki, M. (2001). Donepezil hydrochloride preserves regional cerebral blood flow in patients with Alzheimer's disease. J Nucl Med *42*, 1441-1445.
Nakata, Y., Yasuda, T., Fukaya, M., Yamamori, S., Itakura, M., Nihira, T., Hayakawa, H., Kawanami, A., Kataoka, M., Nagai, M.*, et al.* (2012). Accumulation of α-synuclein triggered by presynaptic dysfunction. J Neurosci *32*, 17186-17196.
Newell, K.L., Hyman, B.T., Growdon, J.H., and Hedley-Whyte, E.T. (1999). Application of the National Institute on Aging (NIA)-Reagan Institute criteria for the neuropathological diagnosis of Alzheimer disease. J Neuropathol Exp Neurol *58*, 1147-1155.
Norton, L.E., Malloy, P.F., and Salloway, S. (2001). The impact of behavioral symptoms on activities of daily living in patients with dementia. Am J Geriatr Psychiatry *9*, 41-48.
Nowak, G., and Schlegel-Zawadzka, M. (1999). Alterations in serum and brain trace element levels after antidepressant treatment: part I. Zinc. Biol Trace Elem Res *67*, 85-92.
O'Brien, B.J., McKeith, I., Ames, D., and Chiu, E. (2006). Dementia with Lewy bodies and Parkinson's disease dementia, 1st edn (London and New York: Taylor & Francis Group).
Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L.*, et al.* (2006). Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. The Journal of neuroscience : the official journal of the Society for Neuroscience *26*, 10129-10140.
Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., and LaFerla, F.M. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron *39*, 409-421.
Ohana, E., Hoch, E., Keasar, C., Kambe, T., Yifrach, O., Hershfinkel, M., and Sekler, I. (2009). Identification of the Zn2+ binding site and mode of operation of a mammalian Zn2+ transporter. J Biol Chem *284*, 17677-17686.
Oinas, M., Polvikoski, T., Sulkava, R., Myllykangas, L., Juva, K., Notkola, I.L., Rastas, S., Niinistö, L., Kalimo, H., and Paetau, A. (2009). Neuropathologic findings of dementia with lewy bodies (DLB) in a population-based Vantaa 85+ study. J Alzheimers Dis *18*, 677-689.
Paik, S.R., Shin, H.J., Lee, J.H., Chang, C.S., and Kim, J. (1999). Copper(II)-induced self-oligomerization of alpha-synuclein. The Biochemical journal *340 (Pt 3)*, 821-828.
Palmiter, R.D., Cole, T.B., Quaife, C.J., and Findley, S.D. (1996). ZnT-3, a putative transporter of zinc into synaptic vesicles. ProcNatlAcadSciUSA *93*, 14934-14939.
Paoletti, P., Ascher, P., and Neyton, J. (1997). High-affinity zinc inhibition of NMDA NR1-NR2A receptors. J Neurosci *17*, 5711-5725.
Paoletti, P., Vergnano, A.M., Barbour, B., and Casado, M. (2009). Zinc at glutamatergic synapses. Neuroscience *158*, 126-136.
Papapetropoulos, S., McCorquodale, D.S., Gonzalez, J., Jean-Gilles, L., and Mash, D.C. (2006). Cortical and amygdalar Lewy body burden in Parkinson's disease patients with visual hallucinations. Parkinsonism Relat Disord *12*, 253-256.

Parkkinen, L., Pirttilä, T., and Alafuzoff, I. (2008). Applicability of current staging/categorization of alpha-synuclein pathology and their clinical relevance. Acta Neuropathol *115*, 399-407.
Parkkinen, L., Pirttilä, T., Tervahauta, M., and Alafuzoff, I. (2005). Widespread and abundant alpha-synuclein pathology in a neurologically unimpaired subject. Neuropathology *25*, 304-314.
Periquet, M., Fulga, T., Myllykangas, L., Schlossmacher, M.G., and Feany, M.B. (2007). Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. J Neurosci *27*, 3338-3346.
Perlmutter, J.D., Braun, A.R., and Sachs, J.N. (2009). Curvature dynamics of alpha-synuclein familial Parkinson disease mutants: molecular simulations of the micelle- and bilayer-bound forms. J Biol Chem *284*, 7177-7189.
Perry, E.K., Haroutunian, V., Davis, K.L., Levy, R., Lantos, P., Eagger, S., Honavar, M., Dean, A., Griffiths, M., McKeith, I.G.*, et al.* (1994). Neocortical cholinergic activities differentiate Lewy body dementia from classical Alzheimer's disease. Neuroreport *5*, 747-749.
Perry, E.K., Kilford, L., Lees, A.J., Burn, D.J., and Perry, R.H. (2003). Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs. Ann Neurol *54*, 235-238.
Perry, E.K., Marshall, E., Kerwin, J., Smith, C.J., Jabeen, S., Cheng, A.V., and Perry, R.H. (1990). Evidence of a monoaminergic-cholinergic imbalance related to visual hallucinations in Lewy body dementia. J Neurochem *55*, 1454-1456.
Pham, E., Crews, L., Ubhi, K., Hansen, L., Adame, A., Cartier, A., Salmon, D., Galasko, D., Michael, S., Savas, J.N.*, et al.* (2010). Progressive accumulation of amyloid-beta oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. FEBS J *277*, 3051-3067.
Pickering-Brown, S.M., Mann, D.M., Bourke, J.P., Roberts, D.A., Balderson, D., Burns, A., Byrne, J., and Owen, F. (1994). Apolipoprotein E4 and Alzheimer's disease pathology in Lewy body disease and in other beta-amyloid-forming diseases. Lancet *343*, 1155.
Piggott, M.A., Marshall, E.F., Thomas, N., Lloyd, S., Court, J.A., Jaros, E., Burn, D., Johnson, M., Perry, R.H., McKeith, I.G.*, et al.* (1999). Striatal dopaminergic markers in dementia with Lewy bodies, Alzheimer's and Parkinson's diseases: rostrocaudal distribution. Brain *122 (Pt 8)*, 1449-1468.
Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R.*, et al.* (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science *276*, 2045-2047.
Port, R.L., and Seybold, K.S. (1995). Hippocampal synaptic plasticity as a biological substrate underlying episodic psychosis. Biol Psychiatry *37*, 318-324.
Pozueta, J., Lefort, R., and Shelanski, M.L. (2012). Synaptic changes in Alzheimer's disease and its models. Neuroscience.
Proctor, D.T., Coulson, E.J., and Dodd, P.R. (2010). Reduction in post-synaptic scaffolding PSD-95 and SAP-102 protein levels in the Alzheimer inferior temporal cortex is correlated with disease pathology. J Alzheimers Dis *21*, 795-811.
Quigley, H., Colloby, S.J., and O'Brien, J.T. (2011). PET imaging of brain amyloid in dementia: a review. Int J Geriatr Psychiatry *26*, 991-999.
Racchi, M., Mazzucchelli, M., Lenzken, S.C., Porrello, E., Lanni, C., and Govoni, S. (2005). Role of acetylcholinesterase inhibitors in the regulation of amyloid beta precursor protein (AbetaPP) metabolism. Chem Biol Interact *157-158*, 335-338.
Raichle, M.E. (1994). Images of the mind: studies with modern imaging techniques. Annu Rev Psychol *45*, 333-356.
Rang, H.P., Dale, M.M., Ritter, J.M., and Moore, P.K. (2003). Pharmacology, 5th edn (Elsevier Churchill Livingstone).
Rapp, M.A., Schnaider-Beeri, M., Grossman, H.T., Sano, M., Perl, D.P., Purohit, D.P., Gorman, J.M., and Haroutunian, V. (2006). Increased hippocampal plaques and tangles in patients with Alzheimer disease with a lifetime history of major depression. Arch Gen Psychiatry *63*, 161-167.

Rapp, M.A., Schnaider-Beeri, M., Wysocki, M., Guerrero-Berroa, E., Grossman, H.T., Heinz, A., and Haroutunian, V. (2011). Cognitive decline in patients with dementia as a function of depression. Am J Geriatr Psychiatry *19*, 357-363.
Rasia-Filho, A.A., Dalpian, F., Menezes, I.C., Brusco, J., Moreira, J.E., and Cohen, R.S. (2012). Dendritic spines of the medial amygdala: plasticity, density, shape, and subcellular modulation by sex steroids. Histol Histopathol *27*, 985-1011.
Reddy, M.S. (2010). Depression: the disorder and the burden. Indian J Psychol Med *32*, 1-2.
Reeve, A.K., Park, T.K., Jaros, E., Campbell, G.R., Lax, N.Z., Hepplewhite, P.D., Krishnan, K.J., Elson, J.L., Morris, C.M., McKeith, I.G.*, et al.* (2012). Relationship between mitochondria and α-synuclein: a study of single substantia nigra neurons. Arch Neurol *69*, 385-393.
Reisberg, B., Sclan, S.G., Franssen, E., Kluger, A., and Ferris, S. (1994). Dementia staging in chronic care populations. Alzheimer Dis Assoc Disord *8 Suppl 1*, S188-205.
Relkin, N.R. (2008). Testing the mettle of PBT2 for Alzheimer's disease. Lancet Neurol *7*, 762-763.
Revesz, T., Holton, J.L., Lashley, T., Plant, G., Frangione, B., Rostagno, A., and Ghiso, J. (2009). Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. Acta Neuropathol *118*, 115-130.
Revuelta, G.J., Rosso, A., and Lippa, C.F. (2008). Neuritic Pathology as a Correlate of Synaptic Loss in Dementia With Lewy Bodies. American Journal of Alzheimer's Disease and Other Dementias *23*, 97-102.
Ridha, B.H., Josephs, K.A., and Rossor, M.N. (2005). Delusions and hallucinations in dementia with Lewy bodies: worsening with memantine. Neurology *65*, 481-482.
Roalf, D.R., Moberg, P.J., Xie, S.X., Wolk, D.A., Moelter, S.T., and Arnold, S.E. (2012). Comparative accuracies of two common screening instruments for classification of Alzheimer's disease, mild cognitive impairment, and healthy aging. Alzheimers Dement.
Roberson, E.D., Scearce-Levie, K., Palop, J.J., Yan, F., Cheng, I.H., Wu, T., Gerstein, H., Yu, G.Q., and Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science *316*, 750-754.
Roberts, B.R., Ryan, T.M., Bush, A.I., Masters, C.L., and Duce, J.A. (2012). The role of metallobiology and amyloid-β peptides in Alzheimer's disease. Journal of neurochemistry *120 Suppl 1*, 149-166.
Rockwell, E., Choure, J., Galasko, D., Olichney, J., and Jeste, D.V. (2000). Psychopathology at initial diagnosis in dementia with Lewy bodies versus Alzheimer disease: comparison of matched groups with autopsy-confirmed diagnoses. Int J Geriatr Psychiatry *15*, 819-823.
Rodda, J., and Carter, J. (2012). Cholinesterase inhibitors and memantine for symptomatic treatment of dementia. BMJ *344*, e2986.
Rodŕiguez-Puertas, R., Pascual, J., and Pazos, A. (1996). Effects of freezing storage time on the density of muscarinic receptors in the human postmortem brain: an autoradiographic study in control and Alzheimer's disease brain tissues. Brain Res *728*, 65-71.
Rogawski, M.A., and Wenk, G.L. (2003). The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. CNS Drug Rev *9*, 275-308.
Rolinski, M., Fox, C., Maidment, I., and McShane, R. (2012). Cholinesterase inhibitors for dementia with Lewy bodies, Parkinson's disease dementia and cognitive impairment in Parkinson's disease. Cochrane Database Syst Rev *3*, CD006504.
Roses, A.D. (2006). On the discovery of the genetic association of Apolipoprotein E genotypes and common late-onset Alzheimer disease. J Alzheimers Dis *9*, 361-366.
Ross, O.A., Toft, M., Whittle, A.J., Johnson, J.L., Papapetropoulos, S., Mash, D.C., Litvan, I., Gordon, M.F., Wszolek, Z.K., Farrer, M.J.*, et al.* (2006). Lrrk2 and Lewy body disease. Annals of neurology *59*, 388-393.
Rybicki, B.A., Johnson, C.C., Uman, J., and Gorell, J.M. (1993). Parkinson's disease mortality and the industrial use of heavy metals in Michigan. Movement disorders : official journal of the Movement Disorder Society *8*, 87-92.

Rüfenacht, P., Güntert, A., Bohrmann, B., Ducret, A., and Döbeli, H. (2005). Quantification of the A beta peptide in Alzheimer's plaques by laser dissection microscopy combined with mass spectrometry. J Mass Spectrom *40*, 193-201.
Sachs, G.S. (2006). A review of agitation in mental illness: burden of illness and underlying pathology. J Clin Psychiatry *67 Suppl 10*, 5-12.
Salazar, G., Falcon-Perez, J.M., Harrison, R., and Faundez, V. (2009). SLC30A3 (ZnT3) oligomerization by dityrosine bonds regulates its subcellular localization and metal transport capacity. PLoSOne *4*, e5896.
Salazar, G., Love, R., Werner, E., Doucette, M.M., Cheng, S., Levey, A., and Faundez, V. (2004). The zinc transporter ZnT3 interacts with AP-3 and it is preferentially targeted to a distinct synaptic vesicle subpopulation. Mol Biol Cell *15*, 575-587.
Samuels, S.C., Brickman, A.M., Burd, J.A., Purohit, D.P., Qureshi, P.Q., and Serby, M. (2004). Depression in autopsy-confirmed dementia with Lewy bodies and Alzheimer's disease. Mt Sinai J Med *71*, 55-62.
Sanacora, G., Treccani, G., and Popoli, M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology *62*, 63-77.
Santacruz, K., Lewis, J., Spires, T., Paulson, J., Kotilinek, L., Ingelsson, M., Guimaraes, A., DeTure, M., Ramsden, M., McGowan, E.*, et al.* (2005). Tau suppression in a neurodegenerative mouse model improves memory function. Science *309*, 476-481.
Santos, C.R., Martinho, A., Quintela, T., and Gonçalves, I. (2012). Neuroprotective and neuroregenerative properties of metallothioneins. IUBMB Life *64*, 126-135.
Saura, C.A., Chen, G., Malkani, S., Choi, S.Y., Takahashi, R.H., Zhang, D., Gouras, G.K., Kirkwood, A., Morris, R.G., and Shen, J. (2005). Conditional inactivation of presenilin 1 prevents amyloid accumulation and temporarily rescues contextual and spatial working memory impairments in amyloid precursor protein transgenic mice. J Neurosci *25*, 6755-6764.
Saura, C.A., Choi, S.Y., Beglopoulos, V., Malkani, S., Zhang, D., Shankaranarayana Rao, B.S., Chattarji, S., Kelleher, R.J., Kandel, E.R., Duff, K.*, et al.* (2004). Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. Neuron *42*, 23-36.
Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K.*, et al.* (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature *400*, 173-177.
Schmitt, U., Tanimoto, N., Seeliger, M., Schaeffel, F., and Leube, R.E. (2009). Detection of behavioral alterations and learning deficits in mice lacking synaptophysin. Neuroscience *162*, 234-243.
Selemon, L.D., Rajkowska, G., and Goldman-Rakic, P.S. (1995). Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. Arch Gen Psychiatry *52*, 805-818; discussion 819-820.
Selkoe, D.J. (1991). The molecular pathology of Alzheimer's disease. Neuron *6*, 487-498.
Sensi, S.L., Paoletti, P., Bush, A.I., and Sekler, I. (2009). Zinc in the physiology and pathology of the CNS. NatRevNeurosci *10*, 780-791.
Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., Brett, F.M., Farrell, M.A., Rowan, M.J., Lemere, C.A.*, et al.* (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med *14*, 837-842.
Shao, C.Y., Mirra, S.S., Sait, H.B., Sacktor, T.C., and Sigurdsson, E.M. (2011). Postsynaptic degeneration as revealed by PSD-95 reduction occurs after advanced Aβ and tau pathology in transgenic mouse models of Alzheimer's disease. Acta neuropathologica *122*, 285-292.
Sharp, S.I., Ballard, C.G., Chen, C.P., and Francis, P.T. (2007). Aggressive behavior and neuroleptic medication are associated with increased number of alpha1-adrenoceptors in patients with Alzheimer disease. Am J Geriatr Psychiatry *15*, 435-437.
Shen, Y.C., Tsai, H.M., Ruan, J.W., Liao, Y.C., Chen, S.F., and Chen, C.H. (2012). Genetic and functional analyses of the gene encoding synaptophysin in schizophrenia. Schizophr Res *137*, 14-19.

Sheng, Z.H., and Cai, Q. (2012). Mitochondrial transport in neurons: impact on synaptic homeostasis and neurodegeneration. Nat Rev Neurosci *13*, 77-93.
Shi, S.H., Hayashi, Y., Petralia, R.S., Zaman, S.H., Wenthold, R.J., Svoboda, K., and Malinow, R. (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science *284*, 1811-1816.
Shim, K.S., and Lubec, G. (2002). Drebrin, a dendritic spine protein, is manifold decreased in brains of patients with Alzheimer's disease and Down syndrome. NeurosciLett *324*, 209-212.
Shinohara, M., Petersen, R.C., Dickson, D.W., and Bu, G. (2013). Brain regional correlation of amyloid-β with synapses and apolipoprotein E in non-demented individuals: potential mechanisms underlying regional vulnerability to amyloid-β accumulation. Acta Neuropathol *125*, 535-547.
Shinoura, N., Midorikawa, A., Onodera, T., Tsukada, M., Yamada, R., Tabei, Y., Itoi, C., Saito, S., and Yagi, K. (2013). Damage to the left ventral, arcuate fasciculus and superior longitudinal fasciculus-related pathways induces deficits in object naming, phonological language function and writing, respectively. Int J Neurosci.
Shirao, T., Kojima, N., Kato, Y., and Obata, K. (1988). Molecular cloning of a cDNA for the developmentally regulated brain protein, drebrin. Brain Res *464*, 71-74.
Shirao, T., Kojima, N., Terada, S., and Obata, K. (1990). Expression of three drebrin isoforms in the developing nervous system. NeurosciResSuppl *13*, S106-S111.
Siew, L.K., Love, S., Dawbarn, D., Wilcock, G.K., and Allen, S.J. (2004). Measurement of pre- and post-synaptic proteins in cerebral cortex: effects of post-mortem delay. J Neurosci Methods *139*, 153-159.
Simard, M., and van Reekum, R. (2001). Dementia with Lewy bodies in Down's syndrome. Int J Geriatr Psychiatry *16*, 311-320.
Simard, M., and van Reekum, R. (2004). The acetylcholinesterase inhibitors for treatment of cognitive and behavioral symptoms in dementia with Lewy bodies. J Neuropsychiatry Clin Neurosci *16*, 409-425.
Simpson, I.A., Carruthers, A., and Vannucci, S.J. (2007). Supply and demand in cerebral energy metabolism: the role of nutrient transporters. J Cereb Blood Flow Metab *27*, 1766-1791.
Simón, A.M., Schiapparelli, L., Salazar-Colocho, P., Cuadrado-Tejedor, M., Escribano, L., López de Maturana, R., Del Río, J., Pérez-Mediavilla, A., and Frechilla, D. (2009). Overexpression of wild-type human APP in mice causes cognitive deficits and pathological features unrelated to Abeta levels. Neurobiol Dis *33*, 369-378.
Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R*., et al.* (2003). alpha-Synuclein locus triplication causes Parkinson's disease. Science *302*, 841.
Sink, K.M., Holden, K.F., and Yaffe, K. (2005). Pharmacological treatment of neuropsychiatric symptoms of dementia: a review of the evidence. JAMA *293*, 596-608.
Smart, T.G., Xie, X., and Krishek, B.J. (1994). Modulation of inhibitory and excitatory amino acid receptor ion channels by zinc. Prog Neurobiol *42*, 393-441.
Smidt, K., and Rungby, J. (2012). ZnT3: a zinc transporter active in several organs. Biometals *25*, 1-8.
Sollner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., and Rothman, J.E. (1993). SNAP receptors implicated in vesicle targeting and fusion. Nature *362*, 318-324.
Soscia, S.J., Kirby, J.E., Washicosky, K.J., Tucker, S.M., Ingelsson, M., Hyman, B., Burton, M.A., Goldstein, L.E., Duong, S., Tanzi, R.E*., et al.* (2010). The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One *5*, e9505.
Spillantini, M.G., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R., and Goedert, M. (1997). Alpha-synuclein in Lewy bodies. Nature *388*, 839-840.
Steinberg, M., Tschanz, J.T., Corcoran, C., Steffens, D.C., Norton, M.C., Lyketsos, C.G., and Breitner, J.C. (2004). The persistence of neuropsychiatric symptoms in dementia: the Cache County Study. Int J Geriatr Psychiatry *19*, 19-26.

Stephan, K.E., Friston, K.J., and Frith, C.D. (2009). Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. Schizophr Bull *35*, 509-527.
Stoltenberg, M., Nejsum, L., Larsen, A., and Danscher, G. (2004). Abundance of Zinc Ions in Synaptic Terminals of mocha Mutant Mice: Zinc Transporter 3 Immunohistochemistry and Zinc Sulphide Autometallography. Journal of Molecular Histology *35*, 141-145.
Strauss, G., and Hauser, H. (1986). Stabilization of lipid bilayer vesicles by sucrose during freezing. Proc Natl Acad Sci U S A *83*, 2422-2426.
Sullivan, K.F. (1988). Structure and utilization of tubulin isotypes. Annu Rev Cell Biol *4*, 687-716.
Sultana, R., Banks, W.A., and Butterfield, D.A. (2010). Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnestic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease. J Neurosci Res *88*, 469-477.
Sultzer, D.L., Mahler, M.E., Mandelkern, M.A., Cummings, J.L., Van Gorp, W.G., Hinkin, C.H., and Berisford, M.A. (1995). The relationship between psychiatric symptoms and regional cortical metabolism in Alzheimer's disease. J Neuropsychiatry Clin Neurosci *7*, 476-484.
Sun, X., Steffens, D.C., Au, R., Folstein, M., Summergrad, P., Yee, J., Rosenberg, I., Mwamburi, D.M., and Qiu, W.Q. (2008). Amyloid-associated depression: a prodromal depression of Alzheimer disease? Arch Gen Psychiatry *65*, 542-550.
Sze, C.I., Troncoso, J.C., Kawas, C., Mouton, P., Price, D.L., and Martin, L.J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. J Neuropathol Exp Neurol *56*, 933-944.
Szewczyk, B., Kubera, M., and Nowak, G. (2011). The role of zinc in neurodegenerative inflammatory pathways in depression. Prog Neuropsychopharmacol Biol Psychiatry *35*, 693-701.
Tabaton, M., and Gambetti, P. (2006). Soluble amyloid-beta in the brain: the scarlet pimpernel. J Alzheimers Dis *9*, 127-132.
Takahashi, H., Sekino, Y., Tanaka, S., Mizui, T., Kishi, S., and Shirao, T. (2003). Drebrin-dependent actin clustering in dendritic filopodia governs synaptic targeting of postsynaptic density-95 and dendritic spine morphogenesis. Journal of Neuroscience *23*, 6586-6595.
Takashima, A. (2009). Amyloid-beta, tau, and dementia. J Alzheimers Dis *17*, 729-736.
Takeda, A. (2000). Movement of zinc and its functional significance in the brain. Brain Res Brain Res Rev *34*, 137-148.
Takeda, A., and Tamano, H. (2009). Insight into zinc signaling from dietary zinc deficiency. Brain Res Rev *62*, 33-44.
Tanji, K., Mori, F., Mimura, J., Itoh, K., Kakita, A., Takahashi, H., and Wakabayashi, K. (2010). Proteinase K-resistant alpha-synuclein is deposited in presynapses in human Lewy body disease and A53T alpha-synuclein transgenic mice. Acta Neuropathol.
Tanzi, R.E., and Bertram, L. (2005). Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell *120*, 545-555.
Tariot, P.N., Farlow, M.R., Grossberg, G.T., Graham, S.M., McDonald, S., and Gergel, I. (2004). Memantine treatment in patients with moderate to severe Alzheimer disease already receiving donepezil: a randomized controlled trial. JAMA *291*, 317-324.
Tassabehji, N.M., Corniola, R.S., Alshingiti, A., and Levenson, C.W. (2008). Zinc deficiency induces depression-like symptoms in adult rats. Physiol Behav *95*, 365-369.
Teffer, K., and Semendeferi, K. (2012). Human prefrontal cortex: evolution, development, and pathology. Prog Brain Res *195*, 191-218.
Tekin, S., and Cummings, J.L. (2002). Frontal-subcortical neuronal circuits and clinical neuropsychiatry: an update. J Psychosom Res *53*, 647-654.
Tekin, S., Fairbanks, L.A., O'Connor, S., Rosenberg, S., and Cummings, J.L. (2001). Activities of daily living in Alzheimer's disease: neuropsychiatric, cognitive, and medical illness influences. Am J Geriatr Psychiatry *9*, 81-86.

Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A., and Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol *30*, 572-580.
Thal, D.R., Rüb, U., Orantes, M., and Braak, H. (2002). Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology *58*, 1791-1800.
Tiraboschi, P., Hansen, L.A., Alford, M., Merdes, A., Masliah, E., Thal, L.J., and Corey-Bloom, J. (2002). Early and widespread cholinergic losses differentiate dementia with Lewy bodies from Alzheimer disease. ArchGenPsychiatry *59*, 946-951.
Tiraboschi, P., Hansen, L.A., Alford, M., Sabbagh, M.N., Schoos, B., Masliah, E., Thal, L.J., and Corey-Bloom, J. (2000). Cholinergic dysfunction in diseases with Lewy bodies. Neurology *54*, 407.
Toft, M., Mata, I.F., Kachergus, J.M., Ross, O.A., and Farrer, M.J. (2005a). LRRK2 mutations and Parkinsonism. Lancet *365*, 1229-1230.
Toft, M., Sando, S.B., Melquist, S., Ross, O.A., White, L.R., Aasly, J.O., and Farrer, M.J. (2005b). LRRK2 mutations are not common in Alzheimer's disease. Mech Ageing Dev *126*, 1201-1205.
Tomic, J.L., Pensalfini, A., Head, E., and Glabe, C.G. (2009). Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction. Neurobiol Dis *35*, 352-358.
Tong, J., Wong, H., Guttman, M., Ang, L.C., Forno, L.S., Shimadzu, M., Rajput, A.H., Muenter, M.D., Kish, S.J., Hornykiewicz, O*., et al.* (2010). Brain alpha-synuclein accumulation in multiple system atrophy, Parkinson's disease and progressive supranuclear palsy: a comparative investigation. Brain *133*, 172-188.
Tremblay, R., Chakravarthy, B., Hewitt, K., Tauskela, J., Morley, P., Atkinson, T., and Durkin, J.P. (2000). Transient NMDA receptor inactivation provides long-term protection to cultured cortical neurons from a variety of death signals. J Neurosci *20*, 7183-7192.
Tröster, A.I. (2008). Neuropsychological characteristics of dementia with Lewy bodies and Parkinson's disease with dementia: differentiation, early detection, and implications for "mild cognitive impairment" and biomarkers. Neuropsychol Rev *18*, 103-119.
Uversky, V.N., Li, J., and Fink, A.L. (2001). Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular NK between Parkinson's disease and heavy metal exposure. The Journal of biological chemistry *276*, 44284-44296.
Valiente-Gabioud, A.A., Torres-Monserrat, V., Molina-Rubino, L., Binolfi, A., Griesinger, C., and Fernández, C.O. (2012). Structural basis behind the interaction of Zn^{2+} with the protein α-synuclein and the Aβ peptide: a comparative analysis. J Inorg Biochem *117*, 334-341.
Valtorta, F., Pennuto, M., Bonanomi, D., and Benfenati, F. (2004). Synaptophysin: leading actor or walk-on role in synaptic vesicle exocytosis? Bioessays *26*, 445-453.
van den Berge, S.A., Kevenaar, J.T., Sluijs, J.A., and Hol, E.M. (2012). Dementia in Parkinson's Disease Correlates with α-Synuclein Pathology but Not with Cortical Astrogliosis. Parkinsons Dis *2012*, 420957.
Varea, E., Castillo-Gómez, E., Gómez-Climent, M.A., Blasco-Ibáñez, J.M., Crespo, C., Martínez-Guijarro, F.J., and Nàcher, J. (2007). Chronic antidepressant treatment induces contrasting patterns of synaptophysin and PSA-NCAM expression in different regions of the adult rat telencephalon. Eur Neuropsychopharmacol *17*, 546-557.
Vekrellis, K., Xilouri, M., Emmanouilidou, E., Rideout, H.J., and Stefanis, L. (2011). Pathological roles of alpha-synuclein in neurological disorders. Lancet Neurol *10*, 1015-1025.
Wakabayashi, K., and Takahashi, H. (1997). Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol *38 Suppl 2*, 2-7.
Walker, R.W., and Walker, Z. (2009). Dopamine transporter single photon emission computerized tomography in the diagnosis of dementia with Lewy bodies. Movement disorders : official journal of the Movement Disorder Society *24 Suppl 2*, S754-759.
Walker, Z., Jaros, E., Walker, R.W., Lee, L., Costa, D.C., Livingston, G., Ince, P.G., Perry, R., McKeith, I., and Katona, C.L. (2007). Dementia with Lewy bodies: a comparison of clinical diagnosis, FP-CIT single

photon emission computed tomography imaging and autopsy. J Neurol Neurosurg Psychiatry *78*, 1176-1181.
Walsh, D.M., Klyubin, I., Fadeeva, J.V., Rowan, M.J., and Selkoe, D.J. (2002). Amyloid-beta oligomers: their production, toxicity and therapeutic inhibition. Biochem Soc Trans *30*, 552-557.
Walsh, D.M., and Selkoe, D.J. (2004). Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron *44*, 181-193.
Wang, C.S., Burke, J.R., Steffens, D.C., Hulette, C.M., Breitner, J.C., and Plassman, B.L. (2009). Twin pairs discordant for neuropathologically confirmed Lewy body dementia. J Neurol Neurosurg Psychiatry *80*, 562-565.
Watson, R., Blamire, A.M., Colloby, S.J., Wood, J.S., Barber, R., He, J., and O'Brien, J.T. (2012). Characterizing dementia with Lewy bodies by means of diffusion tensor imaging. Neurology *79*, 906-914.
Weisenbach, S.L., Boore, L.A., and Kales, H.C. (2012). Depression and cognitive impairment in older adults. Curr Psychiatry Rep *14*, 280-288.
Weisman, D., Cho, M., Taylor, C., Adame, A., Thal, L.J., and Hansen, L.A. (2007). In dementia with Lewy bodies, Braak stage determines phenotype, not Lewy body distribution. Neurology *69*, 356-359.
Welsh, S.W., Corrigan, F.M., and Marian, S. (1996). Language Impairment and Aggression in Alzheimer's Disease. International Journal of Geriatric Psychiatry *11*, 257 - 261.
Whittle, N., Lubec, G., and Singewald, N. (2009). Zinc deficiency induces enhanced depression-like behaviour and altered limbic activation reversed by antidepressant treatment in mice. Amino Acids *36*, 147-158.
Wiedenmann, B., and Franke, W.W. (1985). Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. Cell *41*, 1017-1028.
Wilcox, K.C., Lacor, P.N., Pitt, J., and Klein, W.L. (2011). Aβ oligomer-induced synapse degeneration in Alzheimer's disease. Cell Mol Neurobiol *31*, 939-948.
Wills, J., Jones, J., Haggerty, T., Duka, V., Joyce, J.N., and Sidhu, A. (2010). Elevated tauopathy and alpha-synuclein pathology in postmortem Parkinson's disease brains with and without dementia. Exp Neurol *225*, 210-218.
Winblad, B., and Poritis, N. (1999). Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). Int J Geriatr Psychiatry *14*, 135-146.
Wolozin, B.L., Pruchnicki, A., Dickson, D.W., and Davies, P. (1986). A neuronal antigen in the brains of Alzheimer patients. Science *232*, 648-650.
Wright, J.A., Wang, X., and Brown, D.R. (2009). Unique copper-induced oligomers mediate alpha-synuclein toxicity. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *23*, 2384-2393.
Xekardaki, A., Santos, M., Hof, P., Kövari, E., Bouras, C., and Giannakopoulos, P. (2012). Neuropathological substrates and structural changes in late-life depression: the impact of vascular burden. Acta Neuropathol *124*, 453-464.
Yamane, Y., Sakai, K., and Maeda, K. (2011). Dementia with Lewy bodies is associated with higher scores on the Geriatric Depression Scale than is Alzheimer's disease. Psychogeriatrics *11*, 157-165.
Yamashita, S. (2007). Heat-induced antigen retrieval: mechanisms and application to histochemistry. ProgHistochemCytochem *41*, 141-200.
Yamin, G., Glaser, C.B., Uversky, V.N., and Fink, A.L. (2003). Certain metals trigger fibrillation of methionine-oxidized alpha-synuclein. J Biol Chem *278*, 27630-27635.
Yao, P.J., and Coleman, P.D. (1998). Reduction of O-linked N-acetylglucosamine-modified assembly protein-3 in Alzheimer's disease. J Neurosci *18*, 2399-2411.
Yong, S.W., Yoon, J.K., An, Y.S., and Lee, P.H. (2007). A comparison of cerebral glucose metabolism in Parkinson's disease, Parkinson's disease dementia and dementia with Lewy bodies. European journal

of neurology : the official journal of the European Federation of Neurological Societies *14*, 1357-1362.
Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.M., Iwata, N., Saido, T.C., Maeda, J., Suhara, T., Trojanowski, J.Q., and Lee, V.M. (2007). Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron *53*, 337-351.
Zhang, B., Maiti, A., Shively, S., Lakhani, F., McDonald-Jones, G., Bruce, J., Lee, E.B., Xie, S.X., Joyce, S., Li, C*., et al.* (2005). Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. Proc Natl Acad Sci U S A *102*, 227-231.
Zhang, L.H., Wang, X., Stoltenberg, M., Danscher, G., Huang, L., and Wang, Z.Y. (2008). Abundant expression of zinc transporters in the amyloid plaques of Alzheimer's disease brain. Brain ResBull *77*, 55-60.
Zhang, L.H., Wang, X., Zheng, Z.H., Ren, H., Stoltenberg, M., Danscher, G., Huang, L., Rong, M., and Wang, Z.Y. (2010). Altered expression and distribution of zinc transporters in APP/PS1 transgenic mouse brain. Neurobiol Aging *31*, 74-87.
Zhu, W., Xie, W., Pan, T., Xu, P., Fridkin, M., Zheng, H., Jankovic, J., Youdim, M.B., and Le, W. (2007). Prevention and restoration of lactacystin-induced nigrostriatal dopamine neuron degeneration by novel brain-permeable iron chelators. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *21*, 3835-3844.
Zilles, K., and Amunts, K. (2010). Centenary of Brodmann's map--conception and fate. Nat Rev Neurosci *11*, 139-145.
Zotova, E., Holmes, C., Johnston, D., Neal, J.W., Nicoll, J.A., and Boche, D. (2011). Microglial alterations in human Alzheimer's disease following Aβ42 immunization. Neuropathol Appl Neurobiol *37*, 513-524.
Zuidema, S.U., Buursema, A.L., Gerritsen, M.G., Oosterwal, K.C., Smits, M.M., Koopmans, R.T., and de Jonghe, J.F. (2011). Assessing neuropsychiatric symptoms in nursing home patients with dementia: reliability and Reliable Change Index of the Neuropsychiatric Inventory and the Cohen-Mansfield Agitation Inventory. Int J Geriatr Psychiatry *26*, 127-134.

Demographic and confounding variables

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
A011/06	Control	F	82	43.0	6.4	-	-	0	frequent	-	-	-	6
A047/02	Control	F	87	21.5	6.0	0	0	0	none	-	-	-	10
A048/09	Control	M	81	42.0	6.7	1	1	-	-	-	-	-	3
A049/03	Control	M	79	34.0	6.3	-	-	-	-	-	-	-	9
A063/10	Control	F	90	74.0	6.6	-	1	-	-	-	-	-	2
A133/95	Control	M	85	48.0	7.0	-	-	-	-	-	-	-	17
A134/00	Control	M	86	6.0	6.8	2	1	0	none	0	-	-	12
A136/10	Control	F	89	65.0	6.4	2	1	0	none	-	-	-	2
A153/01	Control	M	71	5.0	6.4	0	0	0	none	0	-	-	11
A170/00	Control	F	68	9.0	6.6	0	0	0	none	0	-	-	12
A185/04	Control	M	80	48.0	6.6	4	2	1	sparse	0	-	-	8
A219/97	Control	F	76	63.0	6.0	0	0	0	none	0	-	-	15
A223/96	Control	M	80	11.0	6.7	0	0	0	none	0	-	-	16
A239/95	Control	F	79	38.0	6.5	3	2	1	sparse	0	-	-	17
A283/96	Control	M	77	29.0	6.5	1	1	0	none	0	-	-	16
A308/09	Control	M	66	52.0	6.7	-	-	-	-	-	-	-	3

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
A31/96	Control	M	70	45.0	6.8	0	0	0	none	0	-	-	16
A316/95	Control	M	80	35.0	6.4	1	1	0	none	0	-	-	17
A320/94	Control	M	77	96.0	6.6	-	-	9	-	-	-	-	18
A33/96	Control	F	96	72.0	6.1	2	1	0	none	0	-	-	16
A346/95	Control	M	85	16.0	6.2	4	2	1	sparse	0	-	-	17
A359/08	Control	F	80	22.0	6.5	-	-	-	-	-	-	-	4
A401/97	Control	M	85	42.0	6.1	3	2	0	none	0	-	-	15
A61/96	Control	M	65	29.0	6.8	0	0	0	none	0	-	-	16
A94/95	Control	F	80	31.0	6.2	1	1	0	none	0	-	-	17
A143/00	PDD	F	89	54.0	6.1	2	1	0	none	0	-	-	10
20020080	PDD	M	70	17.0	6.2	2	1	0	none	6	5	12	9
20030004	PDD	F	69	46.0	6.6	2	1	0	none	11	10	10	9
20030103	PDD	F	73	30.0	5.8	4	2	1	sparse	16	4	10	9
20030111	PDD	M	81	40.0	5.9	2	1	0	none	11	8	9	9
20030134	PDD	M	75	40.0	6.5	3	2	1	sparse	19	2	4	8
20040022	PDD	M	79	30.0	6.8	3	2	1	sparse	15	9	14	8
20040076	PDD	M	76	17.0	6.4	2	1	0	none	12	7	11	8

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
20040105	PDD	M	68	11.0	6.2	5	3	3	frequent	18	6	8	7
20050096	PDD	M	73	31.0	5.8	0	0	0	none	8	5	23	7
20050099	PDD	M	89	64.0	6.0	3	2	1	sparse	9	3	16	12
ST01/01	PDD	F	83	24.0	6.6	-	1	2	moderate	11	4	19	11
ST02/01	PDD	M	83	37.0	6.5	-	1	0	none	6	1	15	11
ST03/01	PDD	M	75	36.0	6.3	-	1	1	sparse	14	1	13	11
ST04/01	PDD	F	85	-	6.8	-	2	3	frequent	18	4	8	11
ST09/02	PDD	M	79	72.0	6.8	-	3	3	frequent	3	1	9	10
ST10/02	PDD	M	82	24.0	6.4	-	1	1	sparse	12	1	3.5	10
ST11/02	PDD	F	73	60.0	6.7	2	1	1	sparse	-	-	-	10
ST12/02	PDD	F	80	28.0	6.3	-	1	0	none	3	1	6	10
ST13/02	PDD	F	81	28.0	6.9	2.0	1	1	sparse	-	-	-	10
ST14/02	PDD	M	78	24.0	6.5	3.0	2	2	moderate	-	-	-	10
ST15/02	PDD	F	88	72.0	5.9	2.0	1	0	none	-	-	-	10
ST16/02	PDD	M	80	26.0	6.6	2.0	1	0	none	-	-	-	10
ST17/02	PDD	M	72	9.0	6.9	1.0	1	0	none	-	-	-	10
ST18/02	PDD	M	79	30.0	6.8	-	1	1	sparse	2	5	16	10

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
ST19/02	PDD	F	84	27.0	6.2	-	1	0	none	6	-	6	10
ST20/02	PDD	F	85	36.0	6.4	-	1	2	moderate	20	2	16	10
ST21/03	PDD	F	83	24.0	6.5	-	1	3	frequent	15	2	26	9
ST22/02	PDD	F	75	24.0	7.2	-	1	3	frequent	6	2	7	9
ST23/03	PDD	F	82	33.0	6.7	-	1	2	moderate	14	2	14	9
ST24/03	PDD	M	88	24.0	6.5	-	2	1	sparse	-	6	15	9
ST25/04	PDD	F	86	24.0	6.3	2	1	1	sparse	-	7	21	8
ST29/04	PDD	F	88	32.0	6.2	-	2	1	sparse	-	-	-	8
ST30/04	PDD	M	86	32.0	6.7	-	0	0	-	-	-	-	8
A014/07	DLB	M	74	20.0	6.9	-	3	0	frequent	18	-	-	19
A028/10	DLB	M	81	85.0	6.6	-	2	0	moderate	14	-	-	19
A035/08	DLB	F	83	14.0	6.2	-	2	0	sparse	18	-	-	21
A040/10	DLB	F	87	33.0	6.1	-	1	0	none	12	-	-	18
A046/07	DLB	M	76	53.0	6.5	-	2	0	moderate	9	-	-	14
A053/09	DLB	M	91	45.0	6.2	5	3	3	frequent	10	-	-	13
A055/09	DLB	F	87	30.0	6.3	5	3	3	frequent	13	-	-	9
A072/09	DLB	F	92	56.0	6.7	5	3	3	frequent	12	-	-	9

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
A084/09	DLB	F	85	31.0	5.9	5	3	3	frequent	-	-	-	8
A092/07	DLB	M	88	17.5	6.5	6	3	3	frequent	18	-	-	8
A109/01	DLB	M	65	5.0	6.6	3	2	1	sparse	19	9	7	7
A148/08	DLB	F	84	13.5	6.1	4	2	2	moderate	11	-	-	7
A162/07	DLB	M	80	25.0	7.3	3	2	1	sparse	7	-	-	6
A190/03	DLB	M	83	38.0	6.2	3	2	1	sparse	16	-	-	5
A196/09	DLB	F	80	28.0	6.6	4	2	1	sparse	20	-	-	5
A204/07	DLB	M	74	18.0	6.7	2	1	0	none	9	-	-	4
A229/05	DLB	M	79	4.0	6.9	3	2	1	sparse	15	-	-	2
A231/06	DLB	F	70	22.5	6.9	3	2	2	moderate	9	-	-	4
A249/06	DLB	M	83	4.0	6.7	4	2	2	moderate	14	3	-	4
A273/05	DLB	M	86	8.0	5.9	2	1	1	sparse	11	4	-	13
A304/06	DLB	F	92	55.0	6.6	3	2	1	sparse	15	9	-	9
A335/08	DLB	M	79	12.3	6.8	1	1	0	none	0	-	-	12
A336/99	DLB	M	69	21.0	7.3	3	2	1	sparse	14	3	-	11
20030007	DLB	F	88	16.0	5.9	3	2	2	moderate	11	8	4	12
20030113	DLB	M	77	65.0	6.0	3	2	1	sparse	17	5	5	11

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
20040034	DLB	F	75	64.0	5.7	6	3	3	frequent	20	3	2	10
20040085	DLB	M	77	29.0	5.7	2	1	2	moderate	18	2.5	2	4
20050030	DLB	F	91	84.0	5.8	5	3	3	frequent	6	7	3	5
20050040	DLB	F	75	78.0	6.0	6	3	3	frequent	16	5	3	2
20060025	DLB	M	76	13.0	6.0	2	1	1	sparse	14	8	7	4
20070009	DLB	M	74	42.0	5.6	4	2	1	sparse	10	8	3	2
20070105	DLB	M	71	8.0	5.7	2	1	1	sparse	19	7	7	5
20080083	DLB	F	80	17.0	5.7	5	3	3	frequent	19	8	6	3
20100575	DLB	M	77	46.0	5.9	3	2	0	none	10	11	7	3
027 93/1089	DLB	F	87	13.0	6.4	5/6	3	3	frequent	-	6	0	3
036 93/1075	DLB	M	85	19.0	6.2	5/6	3	3	frequent	-	10	4	3
051 91/1249	DLB	M	82	80.0	6.4	5/6	3	3	frequent	-	3	0	5
052 94/1224	DLB	M	82	29.0	6.2	3/4	2	2	moderate	-	7	1	11
055 98/1226	DLB	M	81	38.0	6.7	3/4	2	1	sparse	-	9	24	4
106 99/1109	DLB	F	88	34.0	6.8	3/4	2	2	moderate	-	0	0	5
333 08/064	DLB	F	87	24.0	6.2	5/6	3	3	frequent	-	99	99	9
367 08/134	DLB	F	92	96.0	6.5	3/4	2	2	moderate	-	14	0	3

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
383 99/1147	DLB	F	92	60.0	6.0	1/2	1	1	sparse	-	3	0	5
436 03/148	DLB	M	76	70.0	6.2	1/2	1	3	frequent	-	7	5	7
439 00/1140	DLB	M	75	76.0	6.9	3/4	2	2	moderate	-	4	3	6
470 01/156	DLB	M	84	74.0	6.5	3/4	2	3	frequent	-	4	2	6
475 00/1108	DLB	F	85	38.0	6.3	3/4	2	3	frequent	-	2	1	7
495 01/172	DLB	M	86	115.0	6.7	3/4	2	2	moderate	-	6	3	6
550 02/021	DLB	M	77	57.0	6.9	5/6	3	0	none	-	1	1	4
745 08/126	DLB	F	76	96.0	6.5	1/2	1	3	frequent	-	10	10	13
C1007 01/176	DLB	M	82	55.0	6.8	1/2	1	0	none	-	6	9	11
ST26/04	DLB	M	90	48.0	6.5	5	3	3	frequent	-	4	-	8
ST27/04	DLB	M	80	84.0	6.5	5	3	3	frequent	-	7	-	8
ST28/04	DLB	F	88	-	6.6	3	2	2	moderate	-	8	-	8
ST32/05	DLB	F	88	24.0	6.1	6	3	2	moderate	-	10	-	7
A071/09	AD	M	80	10.0	6.3	6	3	-	-	-	9	0	3
A108/09	AD	F	84	24.5	6.7	4	2	-	-	-	8	0	3
A120/09	AD	F	85	79.0	6.3	6	3	-	-	-	16	0	3
A147/10	AD	F	85	20.5	5.9	6	3	-	-	-	13	0	2

Case ID	Diagnosis	Gender	Age	PMD	PH	Braak stage	Coded Braak	CERAD coded	CERAD	cLB score	years of dementia	years of PD	Years in storage
A216/09	AD	F	88	44.0	6.0	5	3	-	-	-	7	0	4
A267/09	AD	F	90	74.0	6.0	5	3	-	-	-	8	0	3
A349/08	AD	F	86	14.0	6.6	6	3	-	-	-	8	0	4
A350/09	AD	F	98	24.0	6.4	4	2	2	moderate	-	11	0	3
A37/09	AD	M	88	29.0	6.5	6	3	-	-	-	12	0	3
A371/08	AD	M	82	70.0	7.1	4	2	-	-	-	9	0	4
A38/11	AD	F	72	67.0	6.0	6	3	-	-	-	9	0	1
A61/09	AD	F	103	12.3	6.4	5	3	-	-	-	13	0	3
A7/10	AD	F	84	30.0	6.1	6	3	-	-	-	8	0	2
A76/09	AD	M	97	18.0	6.2	5	3	3	frequent	-	12	0	3
A8/10	AD	F	98	25.0	6.1	6	3	-	-	-	11	0	2
A92/09	AD	M	88	17.5	6.5	6	3	3	frequent	-	7	0	3

NPI and MMSE values and semi-quantitative scores

Case ID	Diagnosis	Delusions	Hallucinations	Agitation	Depression	Anxiety	Elation	Apathy	Disinhibition	Irritability	Aberrant motor	Sleep	Appitite	Total	MMSE at start	MMSE before death	MMSE decline year	MMSE coded	Hallucination CODED	Persecution CODED	Depression CODED	Agitation CODED
A011/06	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A047/02	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A048/09	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A049/03	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A063/10	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A133/95	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A134/00	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A136/10	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A153/01	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A170/00	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A185/04	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A219/97	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A223/96	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A239/95	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A283/96	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A308/09	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A31/96	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A316/95	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A320/94	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A33/96	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A346/95	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A359/08	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-

Case ID	Diagnosis	Delusions	Hallucinations	Agitation	Depression	Anxiety	Elation	Apathy	Disinhibition	Irritability	Aberrant motor	Sleep	Appitite	Total	MMSE at start	MMSE before death	MMSE decline year	MMSE coded	Hallucination CODED	Persecution CODED	Depression CODED	Agitation CODED
A401/97	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A61/96	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A94/95	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A143/00	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20020080	PDD	3	1	-	1	-	-	-	-	-	-	-	8	13	25	16	9	2	1	2	1	0
20030004	PDD	-	3	-	-	-	-	-	-	-	3	4	-	10	19	19	0	2	2	1	0	0
20030103	PDD	-	-	-	3	3	-	8	-	3	-	-	-	17	27	5	11	1	1	0	3	0
20030111	PDD	-	1	-	-	-	-	4	-	-	-	-	6	11	21	6	7.5	1	1	0	0	0
20030134	PDD	-	-	-	-	-	-	-	-	-	-	-	-	0	27	17	3.3	2	2	0	0	2
20040022	PDD	-	-	-	-	-	-	-	-	-	-	-	-	0	20	0	6.6	1	2	0	1	0
20040076	PDD	-	4	1	-	1	-	12	-	-	4	4	4	30	16	0	8	1	2	1	0	1
20040105	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	22	9	6.5	1	3	2	3	3
20050096	PDD	-	8	1	4	3	-	-	-	-	-	-	-	16	17	19	0	2	3	1	2	1
20050099	PDD	6	-	6	4	-	-	1	-	3	1	8	-	29	23	4	4.8	1	0	2	3	3
ST01/01	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	88	2	1	0	1	0
ST02/01	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	88	2	1	2	2	0
ST03/01	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	88	2	2	2	0	2
ST04/01	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	88	1	0	0	0	3
ST09/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	88	1	2	0	2	1
ST10/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	88	2	0	1	2	3
ST11/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	88	3	3	2	1	2
ST12/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	88	3	0	0	0	0
ST13/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	88	3	1	1	1	1
ST14/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	88	1	0	1	1	0

Case ID	Diagnosis	Delusions	Hallucinations	Agitation	Depression	Anxiety	Elation	Apathy	Disinhibition	Irritability	Aberrant motor	Sleep	Appitite	Total	MMSE at start	MMSE before death	MMSE decline year	MMSE coded	Hallucination CODED	Persecution CODED	Depression CODED	Agitation CODED
ST15/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	88	2	2	0	3	2
ST16/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	88	2	0	2	0	0
ST17/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27	88	3	1	1	2	1
ST18/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	88	1	3	3	3	2
ST19/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	88	-	-	-	-	-
ST20/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	88	1	0	0	3	1
ST21/03	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	88	2	2	2	0	1
ST22/02	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	88	2	2	2	2	0
ST23/03	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	88	1	0	0	1	0
ST24/03	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	88	2	2	0	1	2
ST25/04	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	88	2	1	0	1	1
ST29/04	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	88	1	0	0	3	3
ST30/04	PDD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	88	2	1	0	1	0
A014/07	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	25	24	0.5	2	-	-	-	-
A028/10	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	30	10	6.67	1	-	-	-	-
A035/08	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	29	-	-	-	-	-	-	-
A040/10	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A046/07	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	21	18	0.75	2	-	-	-	-
A053/09	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A055/09	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	25	0	8.33	1	-	-	-	-
A072/09	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A084/09	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A092/07	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A109/01	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	27	12	7.5	2	-	-	-	-

Case ID	Diagnosis	Delusions	Hallucinations	Agitation	Depression	Anxiety	Elation	Apathy	Disinhibition	Irritability	Aberrant motor	Sleep	Appitite	Total	MMSE at start	MMSE before death	MMSE decline year	MMSE coded	Hallucination CODED	Persecution CODED	Depression CODED	Agitation CODED
A148/08	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	24	18	3	2	-	-	-	-
A162/07	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	30	30	0	3	-	-	-	-
A190/03	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A196/09	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A204/07	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	29	0	2.64	1	-	-	-	-
A229/05	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	18	11	7	2	-	-	-	-
A231/06	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A249/06	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	88	1	-	-	-	-
A273/05	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	29	29	0	3	-	-	-	-
A304/06	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	26	-	-	-	-	-	-	-
A335/08	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A336/99	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	28	20	88	2	-	-	-	-
20030007	DLB	1	4	1	1	-	1	8	2	2	-	-	8	28	21	18	1.5	2	3	1	1	2
20030113	DLB	-	-	2	-	-	-	8	1	-	-	-	6	17	20	12	4	2	1	1	1	1
20040034	DLB	6	12	4	2	4	-	4	-	-	6	-	-	38	14	6	4	1	3	3	1	2
20040085	DLB	-	1	4	-	-	-	-	-	-	-	-	3	8	10	88	6	1	2	1	1	2
20050030	DLB	-	-	-	-	-	-	8	-	-	-	-	-	8	25	88	1	3	2	2	1	0
20050040	DLB	-	-	-	-	-	-	-	-	-	-	-	-	0	19	15	4	2	0	0	1	0
20060025	DLB	-	-	-	-	1	-	-	-	-	-	-	8	9	17	88	5	1	3	1	1	2
20070009	DLB	6	4	-	-	-	-	6	-	-	-	-	4	20	26	12	3.5	2	3	2	2	0
20070105	DLB	-	4	4	-	4	-	12	-	-	8	-	12	44	23	1	3.666667	1	3	3	1	2
20080083	DLB	-	-	6	-	-	-	12	-	-	-	-	-	18	11	88	88	9	2	2	0	3
20100575	DLB	-	-	3	1	1	-	8	-	6	-	4	-	23	23	12	2.2	2	1	0	1	2

Case ID	Diagnosis	Delusions	Hallucinations	Agitation	Depression	Anxiety	Elation	Apathy	Disinhibition	Irritability	Aberrant motor	Sleep	Appitite	Total	MMSE at start	MMSE before death	MMSE decline year	MMSE coded	Hallucination CODED	Persecution CODED	Depression CODED	Agitation CODED
027 93/1089	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	24	11	3.7	2	1	-	0	-
036 93/1075	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0	1	1	0	0	0	0
051 91/1249	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	12	6	4	1	0	0	0	0
052 94/1224	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	21	9	2.7	1	2	-	0	-
055 98/1226	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	28	27	0	3	0	0	1	1
106 99/1109	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	29	30	0	3	0	0	0	0
333 08/064	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	21	17	0.42	2	0	-	0	-
367 08/134	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	27	10	1.8	1	1	-	0	-
383 99/1147	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	14	14	-1	2	2	-	-	-
436 03/148	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	22	16	2.2	2	0	1	0	0
439 00/1140	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	23	15	5.3	2	2	0	0	0
470 01/156	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	23	18	3.3	2	1	0	0	0
475 00/1108	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	22	7	15	1	0	2	0	0
495 01/172	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	5	7	0	1	0	1	1	1
550 02/021	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	23	16	7	2	0	0	0	0
745 08/126	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	14	14	14	2	2	-	0	-
C1007 01/176	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	30	25	2.5	3	0	2	0	0
ST26/04	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	17	-	88	1	-	-	-	-
ST27/04	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	22	18	88	2	3	0	0	0
ST28/04	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	27	20	88	2	0	3	3	3
ST32/05	DLB	-	-	-	-	-	-	-	-	-	-	-	-	-	99	-	88	-	-	-	-	-
A071/09	AD	1	-	-	-	-	-	4	1	-	-	-	-	-	23	8	15	1	0	2	1	0
A108/09	AD	-	-	-	-	-	-	12	-	-	8	-	-	-	22	0	5.5	1	0	0	0	0
A120/09	AD	6	-	4	6	-	-	8	1	2	-	6	12	-	10	0	5	1	0	3	3	2

Case ID	Diagnosis	Delusions	Hallucinations	Agitation	Depression	Anxiety	Elation	Apathy	Disinhibition	Irritability	Aberrant motor	Sleep	Appitite	Total	MMSE at start	MMSE before death	MMSE decline year	MMSE coded	Hallucination CODED	Persecution CODED	Depression CODED	Agitation CODED
A147/10	AD	-	-	12	12	12	-	12	12	12	12	-	12	-	21	0	5.25	1	0	0	2	2
A216/09	AD	1	1	1	3	4	-	4	-	-	4	-	12	-	25	16	3	2	1	2	3	0
A267/09	AD	-	-	1	-	2	8	6	3	4	12	-	4	-	11	13	88	2	0	0	3	2
A349/08	AD	-	-	12	8	-	-	4	-	6	-	8	6	-	21	3	4.5	1	0	0	2	3
A350/09	AD	8	-	8	4	-	-	3	-	9	-	3	-	-	20	15	2.5	2	2	3	3	3
A37/09	AD	-	3	1	-	-	-	4	-	-	1	-	3	-	22	17	1	2	2	0	0	1
A371/08	AD	4	2	9	6	-	-	12	6	6	-	6	8	-	21	17	1.33	2	1	2	2	3
A38/11	AD	3	-	12	3	6	-	9	4	4	12	12	8	-	10	0	5	1	0	3	2	3
A61/09	AD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	88	9	-	-	-	-
A7/10	AD	-	-	-	-	-	-	12	-	-	-	-	-	-	12	6	1	1	0	2	0	1
A76/09	AD	-	-	-	-	-	-	-	-	-	-	-	-	-	16	15	0.25	2	0	0	1	0
A8/10	AD	-	-	2	2	-	-	12	-	3	3	-	8	-	10	0	2.5	1	0	2	2	2
A92/09	AD	-	-	3	-	-	-	2	-	3	-	-	4	-	20	19	1	2	0	0	0	2

Semi-quantitative pathology scores

Case ID	Diagnosis	Plq BA9	Plq BA24	Plq BA21	Plq BA40	Tngl BA9	Tngl BA24	Tngl BA21	Tngl BA40	Asyn BA9	Asyn BA24	Asyn BA21	Asyn BA40	Total pathology BA9	Total pathology BA24	Total pathology BA40	Total pathology BA21
A011/06	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A047/02	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A048/09	Control	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
A049/03	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A063/10	Control	0	2	2	0	1	1	1	0	0	0	-	0	1	3	0	-
A133/95	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A134/00	Control	0	0	0	0	0	0	3	0	0	0	-	0	0	0	0	-
A136/10	Control	2	0	3	3	0	0	1	0	0	0	-	-	2	0	-	-
A153/01	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A170/00	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A185/04	Control	1	-	1	2	0	-	0	0	0	-	0	0	1	-	2	1
A219/97	Control	0	0	0	0	0	0	0	0	0	0	-	-	0	0	-	-
A223/96	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A239/95	Control	1	3	1	1	1	1	1	1	0	0	0	0	2	4	2	2
A283/96	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A308/09	Control	1	0	1	1	0	0	0	0	0	0	-	0	1	0	1	-
A31/96	Control	0	-	0	0	0	-	0	0	0	-	0	0	0	-	0	0
A316/95	Control	2	0	2	1	1	0	0	0	0	0	0	0	3	0	1	2
A320/94	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A33/96	Control	0	0	0	0	0	0	0	0	0	0	-	-	0	0	-	-
A346/95	Control	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1
A359/08	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A401/97	Control	0	0	1	0	1	0	1	0	0	0	-	0	1	0	0	-

Case ID	Diagnosis	Plq BA9	Plq BA24	Plq BA21	Plq BA40	Tngl BA9	Tngl BA24	Tngl BA21	Tngl BA40	Asyn BA9	Asyn BA24	Asyn BA21	Asyn BA40	Total pathology BA9	Total pathology BA24	Total pathology BA40	Total pathology BA21
A61/96	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A94/95	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A143/00	PDD	1	1	1	1	1	0	1	1	0	1	0	0	2	2	2	2
20020080	PDD	0	0	1	0	-	0	0	-	1	0	1	1	-	0	-	2
20030004	PDD	1	0	0	0	0	1	1	0	1	3	1	1	2	4	1	2
20030103	PDD	3	2	2	-	1	1	0	0	2	3	1	1	6	6	-	3
20030111	PDD	2	1	1	1	0	1	0	0	1	3	2	0	3	5	1	3
20030134	PDD	2	2	1	1	1	2	1	1	2	3	1	1	5	7	3	3
20040022	PDD	3	1	-	-	1	1	2	1	1	3	1	1	5	5	-	-
20040076	PDD	2	1	1	2	0	1	1	1	1	1	2	2	3	3	5	4
20040105	PDD	2	3	2	2	2	2	2	2	1	2	2	3	5	7	7	6
20050096	PDD	1	3	1	1	1	2	0	0	1	2	2	1	3	7	2	3
20050099	PDD	0	1	1	2	1	1	1	1	2	2	1	1	3	4	4	3
ST01/01	PDD	3	0	1	2	1	0	1	1	1	1	2	1	5	1	4	4
ST02/01	PDD	0	0	0	0	1	0	0	0	0	2	1	1	1	2	1	1
ST03/01	PDD	1	0	0	1	0	0	0	0	1	3	2	0	2	3	1	2
ST04/01	PDD	3	3	1	3	1	1	1	0	2	3	2	0	6	7	3	4
ST09/02	PDD	1	2	-	2	0	0	0	0	0	1	1	0	1	3	2	-
ST10/02	PDD	1	1	0	0	0	0	0	1	1	3	1	0	2	4	1	1
ST11/02	PDD	0	0	1	1	0	0	0	1	0	2	1	0	0	2	2	2
ST12/02	PDD	0	0	-	0	1	0	0	0	0	1	1	0	1	1	0	-
ST13/02	PDD	1	1	1	1	0	0	1	0	1	2	1	0	2	3	1	3
ST14/02	PDD	0	1	1	1	1	1	1	1	1	3	0	0	2	5	2	2
ST15/02	PDD	0	0	0	0	0	0	0	0	0	2	1	1	0	2	1	1
ST16/02	PDD	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0

Case ID	Diagnosis	Plq BA9	Plq BA24	Plq BA21	Plq BA40	Tngl BA9	Tngl BA24	Tngl BA21	Tngl BA40	Asyn BA9	Asyn BA24	Asyn BA21	Asyn BA40	Total pathology BA9	Total pathology BA24	Total pathology BA40	Total pathology BA21
ST17/02	PDD	2	3	1	3	0	0	0	1	0	1	0	0	2	4	4	1
ST18/02	PDD	3	2	1	0	0	1	0	0	0	0	0	0	3	3	0	1
ST19/02	PDD	1	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0
ST20/02	PDD	2	1	1	3	1	1	0	1	2	2	2	1	5	4	5	3
ST21/03	PDD	3	1	1	3	0	0	0	0	0	2	0	0	3	3	3	1
ST22/02	PDD	3	0	1	3	1	0	0	0	0	1	0	0	4	1	3	1
ST23/03	PDD	3	1	1	2	0	0	1	1	1	1	0	1	4	2	4	2
ST24/03	PDD	2	1	1	1	1	0	1	1	3	3	3	2	6	4	4	5
ST25/04	PDD	-	0	0	-	-	1	0	0	0	0	0	0	-	1	-	0
ST29/04	PDD	0	0	0	0	0	0	0	0	1	3	0	0	1	3	0	0
ST30/04	PDD	0	0	0	1	1	1	0	1	0	2	0	1	1	3	3	0
A014/07	DLB	3	3	3	3	3	3	3	3	3	3	3	3	9	9	9	9
A028/10	DLB	1	0	3	0	1	1	1	1	2	3	2	2	4	4	3	6
A035/08	DLB	3	3	1	3	1	1	1	2	3	3	3	3	7	7	8	5
A040/10	DLB	2	2	2	3	1	1	1	1	3	2	2	2	6	5	6	5
A046/07	DLB	2	0	2	0	0	0	1	0	2	2	2	2	4	2	2	5
A053/09	DLB	3	2	3	2	1	3	3	2	0	0	0	0	4	5	4	6
A055/09	DLB	3	3	3	3	2	3	3	2	3	3	2	3	8	9	8	8
A072/09	DLB	1	1	3	1	1	2	2	1	1	3	2	2	3	6	4	7
A084/09	DLB	2	3	3	2	1	3	2	3	2	3	2	1	5	9	6	7
A092/07	DLB	3	1	3	3	1	2	3	1	3	2	3	3	7	5	7	9
A109/01	DLB	2	0	1	1	1	0	1	0	3	3	3	3	6	3	4	5
A148/08	DLB	3	1	2	1	1	1	1	1	1	3	1	1	5	5	3	4
A162/07	DLB	3	0	1	2	0	1	3	0	1	1	0	0	4	2	2	4
A190/03	DLB	0	2	1	1	1	1	1	1	3	1	2	2	4	4	4	4

Case ID	Diagnosis	Plq BA9	Plq BA24	Plq BA21	Plq BA40	Tngl BA9	Tngl BA24	Tngl BA21	Tngl BA40	Asyn BA9	Asyn BA24	Asyn BA21	Asyn BA40	Total pathology BA9	Total pathology BA24	Total pathology BA40	Total pathology BA21
A196/09	DLB	1	1	3	1	1	2	3	1	3	3	3	3	5	6	5	9
A204/07	DLB	0	0	0	0	0	0	0	0	2	2	1	2	2	2	2	1
A229/05	DLB	0	1	2	0	1	0	1	1	3	2	3	3	4	3	4	6
A231/06	DLB	2	2	2	2	1	1	1	1	-	1	1	1	-	4	4	4
A249/06	DLB	1	1	1	1	0	1	1	0	2	3	3	1	3	5	2	5
A273/05	DLB	3	2	3	3	0	0	0	0	2	3	0	1	5	5	4	3
A304/06	DLB	1	1	1	1	1	0	1	1	3	3	3	1	5	4	3	5
A335/08	DLB	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	2
A336/99	DLB	1	0	1	1	1	0	1	1	3	3	3	2	5	3	4	5
20030007	DLB	-	2	1	2	0	1	1	0	3	3	1	1	-	6	3	3
20030113	DLB	3	3	1	1	0	1	0	1	2	-	2	2	5	-	4	3
20040034	DLB	-	1	1	3	3	3	2	3	-	3	2	3	-	7	9	5
20040085	DLB	2	2	2	1	1	1	2	2	2	2	2	1	5	5	4	6
20050030	DLB	2	3	2	3	2	3	1	2	1	1	0	1	5	7	6	3
20050040	DLB	0	0	0	0	2	1	2	1	2	2	-	2	4	3	3	-
20060025	DLB	2	2	2	1	1	1	1	1	1	3	1	1	4	6	3	4
20070009	DLB	2	1	1	1	1	1	1	1	1	2	1	1	4	4	3	3
20070105	DLB	2	2	3	3	1	2	1	1	2	3	2	2	5	7	6	6
20080083	DLB	2	-	1	1	2	2	3	2	2	3	3	1	6	-	4	7
20100575	DLB	0	0	0	0	0	1	0	1	1	2	1	1	1	3	2	1
027 93/1089	DLB	2	-	3	3	1	-	2	1	0	3	3	2	3	-	6	8
036 93/1075	DLB	3	-	3	2	1	-	2	1	2	3	3	2	6	-	5	8
051 91/1249	DLB	3	-	3	3	3	-	3	3	1	3	3	0	7	-	6	9
052 94/1224	DLB	1	0	2	2	0	0	0	0	2	2	3	2	3	2	4	5
055 98/1226	DLB	1	1	1	1	1	2	0	1	1	3	1	0	3	6	2	2

Case ID	Diagnosis	Plq BA9	Plq BA24	Plq BA21	Plq BA40	Tngl BA9	Tngl BA24	Tngl BA21	Tngl BA40	Asyn BA9	Asyn BA24	Asyn BA21	Asyn BA40	Total pathology BA9	Total pathology BA24	Total pathology BA40	Total pathology BA21
106 99/1109	DLB	2	3	2	1	1	3	1	0	0	2	0	0	3	8	1	3
333 08/064	DLB	3	1	3	3	2	2	1	1	0	2	0	0	5	5	4	4
367 08/134	DLB	1	2	2	1	0	2	1	0	0	2	1	1	1	6	2	4
383 99/1147	DLB	1	0	0	0	0	0	0	0	0	2	1	0	1	2	0	1
436 03/148	DLB	0	0	0	0	0	0	0	0	1	2	1	0	1	2	0	1
439 00/1140	DLB	1	2	2	2	1	2	1	0	3	3	3	3	5	7	5	6
470 01/156	DLB	3	0	3	3	1	0	1	1	1	3	2	1	5	3	5	6
475 00/1108	DLB	1	2	3	1	0	1	1	1	0	2	3	1	1	5	3	7
495 01/172	DLB	2	1	2	2	1	1	1	1	1	3	2	1	4	5	4	5
550 02/021	DLB	3	3	3	2	1	3	2	1	1	3	2	1	5	9	4	7
745 08/126	DLB	0	-	0	0	0	0	0	0	2	3	2	1	2	-	1	2
C1007 01/176	DLB	0	0	0	0	0	1	0	0	1	3	1	1	1	4	1	1
ST26/04	DLB	1	1	1	2	1	1	2	0	-	0	-	-	-	2	-	-
ST27/04	DLB	2	2	1	1	1	1	2	1	2	2	3	1	5	5	3	6
ST28/04	DLB	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1
ST32/05	DLB	3	1	1	2	3	2	3	3	1	1	0	1	7	4	6	4
A071/09	AD	3	1	3	3	3	0	3	3	0	0	0	0	6	1	6	6
A108/09	AD	3	1	3	3	2	1	3	3	0	0	0	0	5	2	6	6
A120/09	AD	3	3	3	3	3	3	3	3	0	0	1	0	6	6	6	7
A147/10	AD	3	2	3	3	3	1	3	3	1	0	-	0	7	3	6	-
A216/09	AD	3	2	3	3	3	3	3	3	0	0	0	0	6	5	6	6
A267/09	AD	3	-	3	3	3	1	3	3	0	0	0	0	6	-	6	6
A349/08	AD	3	1	3	3	2	1	1	2	0	0	0	0	5	2	5	4
A350/09	AD	1	0	1	1	2	0	2	2	0	0	0	0	3	0	3	3
A37/09	AD	3	0	3	3	2	0	3	3	0	0	0	0	5	0	6	6

Case ID	Diagnosis	Plq BA9	Plq BA24	Plq BA21	Plq BA40	Tngl BA9	Tngl BA24	Tngl BA21	Tngl BA40	Asyn BA9	Asyn BA24	Asyn BA21	Asyn BA40	Total pathology BA9	Total pathology BA24	Total pathology BA40	Total pathology BA21
A371/08	AD	3	1	3	2	2	0	3	3	0	0	0	0	5	1	5	6
A38/11	AD	3	3	3	3	3	3	3	3	0	2	0	0	6	8	6	6
A61/09	AD	3	3	3	3	2	2	3	3	0	0	0	0	5	5	6	6
A7/10	AD	3	1	2	3	3	3	3	3	0	1	1	1	6	5	7	6
A76/09	AD	3	2	3	2	3	2	3	3	1	2	1	1	7	6	6	7
A8/10	AD	3	2	3	1	2	3	3	2	0	0	0	0	5	5	3	6
A92/09	AD	2	0	3	3	3	0	3	3	0	0	0	0	5	0	6	6

Medication details according to clinical diagnosis.

Controls

		Medication classification according to BNF coding (first medication)	Medication classification according to BNF coding (second medication if applicable)	Medication classification according to BNF coding (3rd medication etc)	Medication classification according to BNF coding (4th etc)	Medication classification according to BNF coding (5th etc)
N	Valid	0	0	0	0	0
	Missing	25	25	25	25	25

PDD

		Medication classification according to BNF coding (first medication)	Medication classification according to BNF coding (second medication if applicable)	Medication classification according to BNF coding (3rd medication etc)	Medication classification according to BNF coding (4th etc)	Medication classification according to BNF coding (5th etc)
N	Valid	31	21	13	5	2
	Missing	3	13	21	29	32

Medication classification according to BNF coding (first medication)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	donezepil	8	23.5	25.8	25.8
	anti-parkinsonism	23	67.6	74.2	100.0
	Total	31	91.2	100.0	
Missing	System	3	8.8		
Total		34	100.0		

Medication classification according to BNF coding (second medication if applicable)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	donezepil	1	2.9	4.8	4.8
	anti-muscarinic for PD	1	2.9	4.8	9.5
	anti-parkinsonism	8	23.5	38.1	47.6
	anti-depressant	9	26.5	42.9	90.5
	anti-psychotic drugs	2	5.9	9.5	100.0
	Total	21	61.8	100.0	
Missing	System	13	38.2		
Total		34	100.0		

Diagnosis = PDD

Medication classification according to BNF coding (3rd medication etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	anti-depressant	6	17.6	46.2	46.2
	anti-psychotic drugs	3	8.8	23.1	69.2
	anxiolytic	2	5.9	15.4	84.6
	hypnotic	2	5.9	15.4	100.0
	Total	13	38.2	100.0	
Missing	System	21	61.8		
Total		34	100.0		

Medication classification according to BNF coding (4th etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	anti-psychotic drugs	3	8.8	60.0	60.0
	hypnotic	2	5.9	40.0	100.0
	Total	5	14.7	100.0	
Missing	System	29	85.3		
Total		34	100.0		

Diagnosis = PDD

Medication classification according to BNF coding (5th etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	hypnotic	2	5.9	100.0	100.0
Missing	System	32	94.1		
Total		34	100.0		

Diagnosis = PDD

DLB

		Medication classification according to BNF coding (first medication)	Medication classification according to BNF coding (second medication if applicable)	Medication classification according to BNF coding (3rd medication etc)	Medication classification according to BNF coding (4th etc)	Medication classification according to BNF coding (5th etc)
N	Valid	38	27	11	3	1
	Missing	17	28	44	52	54

Diagnosis = DLB

Medication classification according to BNF coding (first medication)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	9.00	1	1.8	2.6	2.6
	Tacrine	4	7.3	10.5	13.2
	donezepil	11	20.0	28.9	42.1
	anti-muscarinic for PD	2	3.6	5.3	47.4
	anti-parkinsonism	7	12.7	18.4	65.8
	anti-depressant	3	5.5	7.9	73.7
	anti-psychotic drugs	6	10.9	15.8	89.5
	anxiolytic	2	3.6	5.3	94.7
	hypnotic	2	3.6	5.3	100.0
	Total	38	69.1	100.0	
Missing	System	17	30.9		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (second medication if applicable)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	60.00	1	1.8	3.7	3.7
	Tacrine	4	7.3	14.8	18.5
	anti-parkinsonism	5	9.1	18.5	37.0
	anti-depressant	8	14.5	29.6	66.7
	anti-psychotic drugs	3	5.5	11.1	77.8
	anxiolytic	2	3.6	7.4	85.2
	hypnotic	4	7.3	14.8	100.0
	Total	27	49.1	100.0	
Missing	System	28	50.9		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (3rd medication etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	59.00	1	1.8	9.1	9.1
	Tacrine	1	1.8	9.1	18.2
	Ginkgo	1	1.8	9.1	27.3
	anti-parkinsonism	1	1.8	9.1	36.4
	anti-depressant	2	3.6	18.2	54.5
	82.00	1	1.8	9.1	63.6
	anxiolytic	3	5.5	27.3	90.9
	hypnotic	1	1.8	9.1	100.0
	Total	11	20.0	100.0	
Missing	System	44	80.0		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (4th etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	memantine	1	1.8	33.3	33.3
	anti-depressant	1	1.8	33.3	66.7
	hypnotic	1	1.8	33.3	100.0
	Total	3	5.5	100.0	
Missing	System	52	94.5		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (5th etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	anti-muscarinic for PD	1	1.8	100.0	100.0
Missing	System	54	98.2		
Total		55	100.0		

Diagnosis = DLB

Statistics[a]

		Medication classification according to BNF coding (first medication)	Medication classification according to BNF coding (second medication if applicable)	Medication classification according to BNF coding (3rd medication etc)	Medication classification according to BNF coding (4th etc)	Medication classification according to BNF coding (5th etc)
N	Valid	38	27	11	3	1
	Missing	17	28	44	52	54

Diagnosis = DLB

Medication classification according to BNF coding (first medication)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	9.00	1	1.8	2.6	2.6
	Tacrine	4	7.3	10.5	13.2
	donezepil	11	20.0	28.9	42.1
	anti-muscarinic for PD	2	3.6	5.3	47.4
	anti-parkinsonism	7	12.7	18.4	65.8
	anti-depressant	3	5.5	7.9	73.7
	anti-psychotic drugs	6	10.9	15.8	89.5
	anxiolytic	2	3.6	5.3	94.7
	hypnotic	2	3.6	5.3	100.0
	Total	38	69.1	100.0	
Missing	System	17	30.9		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (second medication if applicable)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	60.00	1	1.8	3.7	3.7
	Tacrine	4	7.3	14.8	18.5
	anti-parkinsonism	5	9.1	18.5	37.0
	anti-depressant	8	14.5	29.6	66.7
	anti-psychotic drugs	3	5.5	11.1	77.8
	anxiolytic	2	3.6	7.4	85.2
	hypnotic	4	7.3	14.8	100.0
	Total	27	49.1	100.0	
Missing	System	28	50.9		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (3rd medication etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	59.00	1	1.8	9.1	9.1
	Tacrine	1	1.8	9.1	18.2
	Ginkgo	1	1.8	9.1	27.3
	anti-parkinsonism	1	1.8	9.1	36.4
	anti-depressant	2	3.6	18.2	54.5
	82.00	1	1.8	9.1	63.6
	anxiolytic	3	5.5	27.3	90.9
	hypnotic	1	1.8	9.1	100.0
	Total	11	20.0	100.0	
Missing	System	44	80.0		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (4th etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	memantine	1	1.8	33.3	33.3
	anti-depressant	1	1.8	33.3	66.7
	hypnotic	1	1.8	33.3	100.0
	Total	3	5.5	100.0	
Missing	System	52	94.5		
Total		55	100.0		

Diagnosis = DLB

Medication classification according to BNF coding (5th etc)[a]

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	anti-muscarinic for PD	1	1.8	100.0	100.0
Missing	System	54	98.2		
Total		55	100.0		

Diagnosis = DLB

Protein values from semi-quantification of Western blotting

CaseID	Drebrin BA9	Betatubulin BA9	PSD95 BA9	SPP BA9	ZnT3 BA9	Betatubulin BA24	PSD95 BA24	SPP BA24	ZnT3 BA24	Betatubulin BA40	PSD95 BA40	SPP BA40	ZnT3 BA40
A011/06	208.01	0.70	0.92	0.61	0.63	1.46	0.42	0.96	0.79	1.02	-	1.00	0.09
A047/02	64.34	1.21	0.99	0.61	0.90	-	-	-	-	1.07	-	1.14	0.17
A048/09	90.32	0.48	0.33	0.67	1.26	1.08	-	0.98	1.43	0.91	0.40	0.71	0.12
A049/03	68.99	1.46	0.84	0.67	1.30	0.60	0.98	1.95	1.09	1.34	0.53	1.25	0.23
A063/10	147.24	0.54	0.28	0.75	0.95	0.86	0.53	0.96	0.38	-	0.23	0.66	0.05
A133/95	-	0.93	0.38	0.61	0.55	-	0.44	0.76	0.38	0.86	0.04	0.80	0.30
A134/00	-	1.49	2.19	0.57	0.65	0.61	0.26	0.77	0.51	0.85	0.98	0.89	-
A136/10	109.30	0.68	0.65	0.92	1.73	1.06	0.40	0.99	1.34	1.14	1.01	1.09	0.71
A153/01	-	0.87	1.02	0.95	0.38	2.04	0.71	0.90	0.47	0.82	2.01	1.13	0.49
A170/00	-	0.59	2.25	0.60	0.29	0.48	0.24	0.77	0.28	0.85	0.98	0.76	0.23
A185/04	85.70	0.79	1.31	0.60	0.25	1.50	-	0.80	0.37	0.80	0.82	1.15	1.12
A219/97	-	-	0.06	0.26	0.08	-	-	-	-	1.05	0.85	0.66	0.61
A223/96	83.59	1.08	0.79	-	0.51	1.37	-	0.87	0.31	1.22	2.00	1.66	0.53
A239/95	50.16	-	0.74	0.75	0.36	1.86	0.23	0.84	0.33	0.86	0.87	0.80	1.30
A283/96	54.17	1.28	0.69	0.75	0.31	0.95	0.44	0.54	0.48	1.38	0.82	0.92	0.43
A308/09	90.68	0.47	0.71	0.66	1.24	0.63	-	0.99	0.86	0.87	0.25	-	0.16
A31/96	-	0.75	1.81	0.63	0.77	0.43	0.53	0.62	0.39	0.80	0.83	0.63	0.41
A316/95	55.85	0.77	0.42	1.24	0.74	0.50	0.72	0.53	0.24	0.90	1.28	0.96	3.68
A320/94	95.48	1.11	0.41	0.86	0.59	-	-	0.88	0.42	0.84	0.64	1.40	-
A33/96	-	1.02	0.24	0.62	0.26	0.77	0.43	0.48	0.27	1.01	0.37	0.96	0.46
A346/95	60.84	0.98	0.60	0.83	0.59	0.58	0.17	0.45	0.31	1.18	0.83	0.61	0.48
A359/08	85.92	1.41	0.70	0.97	1.44	0.68	-	0.73	-	0.90	0.56	-	0.10
A401/97	94.77	0.76	1.60	0.64	0.73	0.98	0.22	0.37	0.12	0.99	0.69	0.77	0.47
A61/96	-	-	-	-	-	0.62	0.24	0.70	-	0.80	0.90	0.78	1.30
A94/95	126.69	0.70	0.93	0.77	0.80	0.90	0.85	0.71	0.41	0.94	1.05	0.84	-

CaseID	Drebrin BA9	Betatubulin BA9	PSD95 BA9	SPP BA9	ZnT3 BA9	Betatubulin BA24	PSD95 BA24	SPP BA24	ZnT3 BA24	Betatubulin BA40	PSD95 BA40	SPP BA40	ZnT3 BA40
A143/00	-	0.75	0.36	0.86	0.20	-	-	-	-	-	-	-	-
20020080	253.18	0.75	0.44	0.70	0.45	1.02	0.65	0.73	0.35	1.69	1.24	1.33	0.71
20030004	161.53	0.53	0.32	0.48	0.51	0.89	0.51	0.76	0.47	1.22	0.78	0.94	0.53
20030103	-	0.83	0.19	0.91	0.24	1.20	0.78	0.75	0.50	0.82	0.71	0.85	-
20030111	-	-	-	-	-	0.82	0.27	0.67	0.33	0.79	0.27	0.65	0.16
20030134	271.98	1.60	0.34	0.30	0.22	0.65	0.38	0.57	0.35	0.57	1.11	0.51	0.40
20040022	248.23	1.04	0.33	0.75	0.37	0.90	0.47	0.57	0.34	0.75	-	0.74	0.75
20040076	32.88	0.64	0.85	0.78	0.44	1.06	1.60	0.49	0.57	1.42	-	0.75	0.48
20040105	97.47	0.49	0.51	0.36	0.25	1.40	1.50	0.52	0.59	1.56	-	0.86	0.29
20050096	79.11	1.05	0.47	-	0.22	0.93	0.87	0.44	0.37	1.02	1.02	1.02	0.99
20050099	137.81	1.03	0.27	0.88	0.50	0.83	0.66	0.58	0.26	0.70	0.50	0.73	0.19
ST01/01	-	0.72	0.61	0.51	0.29	0.47	0.18	0.73	0.54	1.20	0.41	0.99	0.18
ST02/01	106.76	0.74	0.36	0.44	0.29	0.79	0.59	0.78	0.48	1.04	0.95	0.82	0.27
ST03/01	136.37	0.54	0.25	0.45	0.57	0.91	0.16	1.68	0.17	0.70	0.59	1.00	0.45
ST04/01	92.59	0.61	0.47	0.60	0.34	0.93	0.45	1.79	0.35	1.09	-	0.73	0.22
ST09/02	91.36	0.57	0.41	0.37	0.33	0.81	0.04	2.60	0.21	1.06	0.30	0.70	0.34
ST10/02	118.79	0.67	0.49	0.30	0.29	0.84	0.14	0.69	0.21	0.92	1.08	1.16	0.79
ST11/02	58.24	0.61	0.51	0.33	0.31	0.91	0.36	0.68	0.20	1.14	0.93	1.20	0.37
ST12/02	80.49	0.83	0.35	0.32	-	0.99	0.63	-	0.52	1.06	1.22	0.93	0.18
ST13/02	111.90	0.69	0.18	0.29	0.41	0.50	0.34	0.61	0.42	0.89	0.61	0.83	0.45
ST14/02	78.99	0.62	0.39	0.32	0.09	0.92	0.72	1.14	0.14	0.63	-	1.07	0.09
ST15/02	134.36	0.53	0.40	0.30	0.09	0.63	0.10	0.44	0.10	1.10	0.48	1.38	0.06
ST16/02	118.24	0.49	0.52	0.60	0.52	0.76	0.54	0.85	0.40	0.60	0.45	0.84	0.31
ST17/02	92.20	0.68	0.74	0.62	0.31	0.77	0.57	0.64	0.27	-	1.06	0.91	0.36
ST18/02	96.13	0.71	0.42	0.52	0.17	1.16	0.42	0.88	0.72	0.97	1.50	0.96	0.87
ST19/02	-	0.75	0.28	0.22	0.13	0.36	0.31	0.59	0.29	0.50	1.40	0.94	0.49
ST20/02	-	0.70	0.96	0.34	0.29	0.45	0.44	0.75	0.36	1.40	1.27	0.82	0.29

CaseID	Drebrin BA9	Betatubulin BA9	PSD95 BA9	SPP BA9	ZnT3 BA9	Betatubulin BA24	PSD95 BA24	SPP BA24	ZnT3 BA24	Betatubulin BA40	PSD95 BA40	SPP BA40	ZnT3 BA40
ST21/03	-	0.53	0.15	0.29	0.12	0.96	0.53	0.88	0.22	1.32	1.15	0.80	0.17
ST22/02	-	1.78	-	0.55	0.41	0.44	0.21	0.54	0.48	0.98	1.21	0.94	0.43
ST23/03	-	0.54	0.25	0.54	0.59	0.58	0.25	0.55	0.44	1.03	0.43	0.93	0.33
ST24/03	163.76	0.68	0.40	0.55	-	0.90	1.24	0.75	0.30	0.79	-	0.68	0.24
ST25/04	-	0.62	0.42	0.45	0.20	0.41	0.49	0.61	0.35	1.12	0.86	1.39	-
ST29/04	-	0.53	0.32	0.35	0.14	0.37	0.52	0.38	0.65	1.43	1.09	0.92	0.55
ST30/04	-	0.60	0.16	-	0.17	1.11	0.07	-	0.21	0.44	0.81	0.88	0.40
A014/07	-	0.75	0.62	0.85	0.55	-	-	-	-	-	-	-	-
A028/10	-	1.09	-	-	-	-	-	-	-	-	-	-	-
A035/08	-	0.70	0.75	-	0.49	-	0.62	-	-	-	-	-	-
A040/10	-	-	-	-	-	-	-	-	-	-	-	-	-
A046/07	-	0.85	0.47	0.68	-	-	-	-	-	-	1.36	-	0.39
A053/09	-	0.98	0.24	0.70	-	0.69	-	0.88	-	-	-	-	-
A055/09	-	0.93	0.33	-	0.92	0.59	-	0.59	-	-	-	-	-
A072/09	-	0.53	-	-	-	-	-	-	-	-	-	-	-
A084/09	-	-	0.71	0.56	1.06	-	-	-	-	-	-	-	-
A092/07	-	1.13	0.57	-	0.47	-	0.52	-	-	-	-	-	-
A109/01	-	0.76	0.81	-	0.56	1.50	1.06	0.40	-	-	-	-	-
A148/08	-	-	-	-	-	0.96	-	0.48	0.27	-	-	-	0.26
A162/07	-	-	-	-	-	0.99	-	0.46	-	-	-	-	0.33
A190/03	-	0.59	0.51	0.78	0.48	-	0.87	-	-	-	-	-	-
A196/09	-	-	0.67	-	-	-	-	-	-	-	-	-	-
A204/07	-	1.41	0.73	-	0.45	1.35	1.22	0.76	0.86	-	-	-	-
A229/05	-	0.55	0.67	0.57	0.49	-	0.63	-	-	-	-	-	-
A231/06	-	0.34	0.20	-	0.17	1.49	0.35	0.78	-	-	-	-	-
A249/06	-	-	-	-	-	1.68	-	0.64	1.02	-	-	-	0.41
A273/05	-	0.87	0.88	-	0.61	1.16	0.58	0.65	-	-	1.12	-	0.18

CaseID	Drebrin BA9	Betatubulin BA9	PSD95 BA9	SPP BA9	ZnT3 BA9	Betatubulin BA24	PSD95 BA24	SPP BA24	ZnT3 BA24	Betatubulin BA40	PSD95 BA40	SPP BA40	ZnT3 BA40
A304/06	-	-	0.62	-	0.54	1.04	0.62	0.60	-	-	-	-	-
A335/08	-	0.65	0.62	-	0.35	1.05	0.52	0.63	0.35	-	-	-	0.30
A336/99	-	0.68	0.35	0.55	0.40	-	-	-	-	-	0.54	-	0.32
20030007	73.53	0.79	0.72	1.19	0.26	0.83	0.45	0.85	0.35	0.82	0.55	1.07	0.37
20030113	129.80	2.18	0.16	0.68	0.33	0.86	0.55	0.93	0.25	0.84	0.63	0.80	0.18
20040034	77.68	1.49	0.42	0.71	0.21	-	0.31	0.72	0.10	0.83	0.33	1.07	0.14
20040085	257.26	-	0.36	1.18	0.37	0.66	0.30	0.60	0.20	0.74	0.26	0.92	0.20
20050030	76.20	0.73	0.46	1.29	0.25	-	0.43	1.08	0.13	0.89	0.27	0.75	0.08
20050040	106.23	-	0.31	0.67	0.23	-	0.57	1.03	0.26	1.00	0.24	1.04	0.13
20060025	-	-	-	-	-	-	0.70	-	-	0.90	0.81	0.81	0.35
20070009	167.98	1.08	0.42	0.72	0.37	0.87	1.01	0.94	0.29	0.57	0.40	0.96	0.17
20070105	166.73	0.83	0.87	1.18	0.34	0.83	0.52	0.81	0.23	0.90	0.95	1.01	0.32
20080083	177.46	1.08	0.53	0.89	0.48	0.70	0.39	0.67	0.28	1.04	0.47	0.88	0.29
20100575	122.08	1.01	0.25	1.34	0.55	0.95	0.29	0.81	0.43	0.88	0.36	0.79	0.26
027 93/1089	129.68	0.78	0.38	0.46	0.19	1.03	0.38	0.73	0.15	0.73	0.68	-	0.48
036 93/1075	-	-	-	-	-	1.61	1.05	1.13	0.19	0.85	-	0.89	-
051 91/1249	88.86	0.94	0.18	0.70	0.17	1.33	0.59	1.02	0.54	0.58	0.44	0.56	0.19
052 94/1224	65.20	0.86	0.69	0.54	0.32	0.84	0.50	0.90	0.33	0.44	0.83	0.82	0.26
055 98/1226	-	0.97	0.32	0.57	0.19	1.07	1.07	0.96	0.54	-	-	-	-
106 99/1109	100.64	1.31	0.89	1.29	0.36	1.09	2.19	-	0.51	0.86	0.10	0.60	0.13
333 08/064	111.29	0.88	0.69	0.81	0.20	1.07	0.49	0.70	0.15	0.84	0.27	0.58	0.19
367 08/134	-	1.03	0.28	1.05	0.20	0.94	0.32	0.54	0.12	0.71	0.72	-	0.15
383 99/1147	-	1.21	0.78	0.62	0.34	-	0.78	-	0.25	1.43	0.53	0.91	0.31
436 03/148	-	0.81	0.72	0.79	0.47	-	0.81	0.94	0.39	0.75	0.73	-	0.50
439 00/1140	-	0.95	-	1.02	0.51	0.78	0.26	0.89	0.30	0.81	0.36	0.65	0.15
470 01/156	-	0.98	0.28	0.72	0.18	0.73	-	0.81	0.33	0.49	0.57	0.74	0.31
475 00/1108	60.09	0.74	0.42	0.84	0.30	0.98	-	0.61	0.12	0.78	-	0.78	0.09

CaseID	Drebrin BA9	Betatubulin BA9	PSD95 BA9	SPP BA9	ZnT3 BA9	Betatubulin BA24	PSD95 BA24	SPP BA24	ZnT3 BA24	Betatubulin BA40	PSD95 BA40	SPP BA40	ZnT3 BA40
495 01/172	-	0.82	0.32	0.82	0.57	0.73	0.38	0.71	0.18	1.23	0.69	0.82	0.18
550 02/021	54.34	1.11	0.33	0.71	0.37	1.09	0.50	1.14	0.23	0.87	0.43	0.70	0.24
745 08/126	-	0.69	0.38	0.74	0.50	1.24	0.30	1.41	0.32	0.31	0.69	0.66	0.31
C1007 01/176	77.14	0.91	0.33	0.63	0.12	1.41	0.54	1.11	0.39	0.59	1.21	0.86	0.29
ST26/04	109.91	0.68	0.91	0.72	0.52	0.86	0.42	0.86	0.18	0.81	-	0.96	0.35
ST27/04	-	0.66	0.37	0.78	0.39	0.84	0.99	0.94	0.85	0.94	0.20	-	0.42
ST28/04	-	0.53	0.36	0.64	0.27	0.44	0.39	0.70	0.60	0.77	0.26	0.95	-
ST32/05	-	0.57	0.40	0.35	0.17	0.46	0.22	0.60	0.21	-	0.37	0.91	0.11
A071/09	53.15	1.04	0.48	1.21	0.69	0.99	0.58	0.82	0.67	0.53	0.22	0.57	0.08
A108/09	-	0.78	0.53	0.71	0.48	0.63	0.34	0.74	0.52	0.74	0.55	1.01	0.38
A120/09	114.46	0.84	-	0.77	0.34	0.63	0.43	0.84	0.09	0.58	0.38	0.79	0.17
A147/10	84.42	0.74	0.68	1.57	0.16	0.85	0.90	0.79	0.06	0.22	0.28	0.31	0.23
A216/09	64.90	0.34	-	1.53	0.22	0.73	0.45	0.65	0.32	0.47	-	0.52	0.07
A267/09	117.32	0.78	0.32	1.37	0.13	0.71	0.70	0.51	0.08	0.57	0.37	0.50	-
A349/08	53.41	-	0.32	1.66	0.23	0.99	0.81	-	0.21	0.39	0.32	0.59	0.28
A350/09	68.75	0.94	0.40	1.46	0.45	1.21	-	1.17	0.52	0.40	-	0.60	0.38
A37/09	66.23	0.85	1.07	0.53	0.50	0.96	1.92	1.06	0.45	0.43	-	0.71	-
A371/08	90.02	0.71	0.32	1.44	0.52	0.69	0.44	0.68	0.48	0.49	0.29	0.58	0.33
A38/11	132.53	0.77	0.68	1.47	0.18	0.72	1.08	0.71	0.17	0.32	0.32	0.44	0.29
A61/09	74.92	0.95	0.66	0.84	0.44	0.91	0.82	0.88	0.07	0.69	-	1.37	0.47
A7/10	32.64	0.86	0.93	0.57	0.51	0.85	1.17	1.03	0.25	1.12	-	0.33	0.17
A76/09	112.82	0.88	1.10	1.28	0.77	0.66	0.68	0.87	0.29	0.41	0.90	0.60	0.56
A8/10	77.41	0.74	-	1.36	0.29	0.70	-	0.84	0.25	0.43	0.50	0.51	0.19
A92/09	123.83	1.09	1.30	0.95	0.58	0.94	0.93	1.10	0.71	0.47	-	0.93	0.33

Residual and normalised protein values in BA9

CaseID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Normalised Btub	Residual PSD95	Residual SPP	Residual ZnT3
A011/06	Control	0.7	0.92	0.61	0.63	0.85	0.29	-0.03	0.29
A047/02	Control	1.21	0.99	0.61	0.9	1.08	0.24	-0.20	0.45
A048/09	Control	0.48	0.33	0.67	1.26	0.68	-0.16	0.12	0.48
A049/03	Control	1.46	0.84	0.67	1.3	1.16	0.21	-0.26	0.5
A063/10	Control	0.54	0.28	0.75	0.95	0.73	-0.11	0.10	0.47
A133/95	Control	0.93	0.38	0.61	0.55	0.97	-0.08	0.03	0.12
A134/00	Control	1.49	2.19	0.57	0.65	1.17	0.53	-0.29	0.2
A136/10	Control	0.68	0.65	0.92	1.73	0.83	0.22	0.09	0.73
A153/01	Control	0.87	1.02	0.95	0.38	0.94	0.19	0.15	-0.04
A170/00	Control	0.59	2.25	0.6	0.29	0.77	0.55	0.13	-0.04
A185/04	Control	0.79	1.31	0.6	0.25	0.9	0.46	-0.06	-0.22
A219/97	Control	-	-	0.26	0.08	-	-	-	-0.6
A223/96	Control	1.08	0.79	-	0.51	1.03	0.1	-	0.09
A239/95	Control	-	0.74	0.75	0.36	-	0.17	-	0.05
A283/96	Control	1.28	0.69	0.75	0.31	1.11	0.11	-0.04	-0.12
A308/09	Control	0.47	0.71	0.66	1.24	0.67	0.21	0.13	0.48
A31/96	Control	0.75	1.81	0.63	0.77	0.88	0.59	0.12	0.27
A316/95	Control	0.77	0.42	1.24	0.74	0.89	-0.08	0.42	0.25
A320/94	Control	1.11	0.41	0.86	0.59	1.05	0.13	0.12	0.15
A33/96	Control	1.02	0.24	0.62	0.26	1.01	-0.19	-0.02	-0.09
A346/95	Control	0.98	0.6	0.83	0.59	0.99	0	0.14	0.15
A359/08	Control	1.41	0.7	0.97	1.44	1.15	0.09	-0.17	0.65
A401/97	Control	0.76	1.6	0.64	0.73	0.88	0.52	0.10	0.25

CaseID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Normalised Btub	Residual PSD95	Residual SPP	Residual ZnT3
A61/96	Control	-	-	-	-	-	-	-	-
A94/95	Control	0.7	0.93	0.77	0.8	0.85	0.25	0.25	0.4
20020080	PDD	0.75	0.44	0.7	0.45	0.88	-0.13	0.06	0.04
20030004	PDD	0.53	0.32	0.48	0.51	0.72	-0.16	0.03	0.2
20030103	PDD	0.83	0.19	0.91	0.24	0.92	-0.45	0.12	-0.13
20030111	PDD	-	-	-	-	-	-	-	-
20030134	PDD	1.6	0.34	0.3	0.22	1.2	-0.16	-0.65	-0.27
20040022	PDD	1.04	0.33	0.75	0.37	1.02	-0.21	-0.08	-0.05
20040076	PDD	0.64	0.85	0.78	0.44	0.81	0.16	0.15	0.03
20040105	PDD	0.49	0.51	0.36	0.25	0.69	-0.09	-0.07	-0.22
20050096	PDD	1.05	0.47	-	0.22	1.02	-0.05	-	-0.27
20050099	PDD	1.03	0.27	0.88	0.5	1.01	-0.17	-0.02	0.08
A143/00	PDD	0.75	0.36	0.86	0.2	0.88	-0.08	0.19	-0.21
ST01/01	PDD	0.72	0.61	0.51	0.29	0.86	0.04	-0.04	-0.04
ST02/01	PDD	0.74	0.36	0.44	0.29	0.87	-0.14	-0.11	-0.15
ST03/01	PDD	0.54	0.25	0.45	0.57	0.73	-0.3	0.03	0.14
ST04/01	PDD	0.61	0.47	0.6	0.34	0.79	-	0.10	0.02
ST09/02	PDD	0.57	0.41	0.37	0.33	0.76	0.04	-0.09	-0.1
ST10/02	PDD	0.67	0.49	0.3	0.29	0.83	-0.06	-0.25	-0.15
ST11/02	PDD	0.61	0.51	0.33	0.31	0.79	0.09	-0.17	-0.02
ST12/02	PDD	0.83	0.35	0.32	-	0.92	-0.19	-0.32	-
ST13/02	PDD	0.69	0.18	0.29	0.41	0.84	-0.48	-0.28	0.11
ST14/02	PDD	0.62	0.39	0.32	0.09	0.79	-0.16	-0.19	-0.66
ST15/02	PDD	0.53	0.4	0.3	0.09	0.72	0.03	-0.15	-0.55
ST16/02	PDD	0.49	0.52	0.6	0.52	0.69	-0.02	0.18	0.1

CaseID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Normalised Btub	Residual PSD95	Residual SPP	Residual ZnT3
ST17/02	PDD	0.68	0.74	0.62	0.31	0.83	0.07	0.05	-0.12
ST18/02	PDD	0.71	0.42	0.52	0.17	0.85	-0.1	-0.04	-0.39
ST19/02	PDD	0.75	0.28	0.22	0.13	0.88	-0.29	-0.44	-0.39
ST20/02	PDD	0.7	0.96	0.34	0.29	0.85	0.28	-0.22	-0.04
ST21/03	PDD	0.53	0.15	0.29	0.12	0.72	-0.57	-0.18	-0.43
ST22/02	PDD	1.78	-	0.55	0.41	1.25	-	-0.43	0.11
ST23/03	PDD	0.54	0.25	0.54	0.59	0.73	-0.32	0.08	0.26
ST24/03	PDD	0.68	0.4	0.55	-	0.83	-0.15	-0.01	-
ST25/04	PDD	0.62	0.42	0.45	0.2	0.79	-0.12	-0.08	-0.21
ST29/04	PDD	0.53	0.32	0.35	0.14	0.72	-0.21	-0.12	-0.36
ST30/04	PDD	0.6	0.16	-	0.17	0.78	-0.51	-	-0.39
027 93/1089	DLB	0.78	0.38	0.46	0.19	0.89	-0.21	0.01	-0.23
036 93/1075	DLB	-	-	-	-	-	-	-	-
051 91/1249	DLB	0.94	0.18	0.7	0.17	0.97	-0.28	0.15	-0.39
052 94/1224	DLB	0.86	0.69	0.54	0.32	0.93	0.11	0.03	-0.11
055 98/1226	DLB	0.97	0.32	0.57	0.19	0.99	-0.19	-0.07	-0.34
106 99/1109	DLB	1.31	0.89	1.29	0.36	1.12	0.24	0.14	0.05
20030007	DLB	0.79	0.72	1.19	0.26	0.9	0.08	0.26	-0.09
20030113	DLB	2.18	0.16	0.68	0.33	1.34	-0.39	-0.43	-0.1
20040034	DLB	1.49	0.42	0.71	0.21	1.17	0.02	-0.26	-0.18
20040085	DLB	-	0.36	1.18	0.37	-	-0.17	-	-0.05
20050030	DLB	0.73	0.46	1.29	0.25	0.86	0.14	0.29	-0.11

CaseID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Normalised Btub	Residual PSD95	Residual SPP	Residual ZnT3
20050040	DLB	-	0.31	0.67	0.23	-	-0.06	-	-0.15
20060025	DLB	-	-	-	-	-	-	-	-
20070009	DLB	1.08	0.42	0.72	0.37	1.03	-0.06	-0.17	-0.05
20070105	DLB	0.83	0.87	1.18	0.34	0.92	0.13	0.16	-0.08
20080083	DLB	1.08	0.53	0.89	0.48	1.03	-0.05	-0.09	0.17
20100575	DLB	1.01	0.25	1.34	0.55	1	-0.27	0.08	0.12
333 08/064	DLB	0.88	0.69	0.81	0.2	0.94	0.09	-0.04	-0.21
367 08/134	DLB	1.03	0.28	1.05	0.2	1.01	-0.03	0.00	-0.21
383 99/1147	DLB	1.21	0.78	0.62	0.34	1.08	0.28	-0.15	0.02
436 03/148	DLB	0.81	0.72	0.79	0.47	0.91	0.28	0.07	0.06
439 00/1140	DLB	0.95	-	1.02	0.51	0.98	-	0.16	0.09
470 01/156	DLB	0.98	0.28	0.72	0.18	0.99	-0.11	-0.02	-0.36
475 00/1108	DLB	0.74	0.42	0.84	0.3	0.87	-0.07	0.18	-0.03
495 01/172	DLB	0.82	0.32	0.82	0.57	0.91	0.1	0.11	0.14
550 02/021	DLB	1.11	0.33	0.71	0.37	1.05	-0.11	-0.10	-0.05
745 08/126	DLB	0.69	0.38	0.74	0.5	0.84	0.1	0.02	0.19
A014/07	DLB	0.75	0.62	0.85	0.55	0.88	0.03	0.07	0.12
A028/10	DLB	1.09	-	-	-	1.04	-	-	-
A035/08	DLB	0.7	0.75	-	0.49	0.85	0.09	-	0.18
A040/10	DLB	-	-	-	-	-	-	-	-
A046/07	DLB	0.85	0.47	0.68	-	0.93	0.03	-0.09	-
A053/09	DLB	0.98	0.24	0.7	-	0.99	-0.29	-0.17	-
A055/09	DLB	0.93	0.33	-	0.92	0.97	-0.21	-	0.46

CaseID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Normalised Btub	Residual PSD95	Residual SPP	Residual ZnT3
A072/09	DLB	0.53	-	-	-	0.72	-	-	-
A084/09	DLB	-	0.71	0.56	1.06	-	0.13	-	0.52
A092/07	DLB	1.13	0.57	-	0.47	1.05	-0.02	-	0.06
A109/01	DLB	0.76	0.81	-	0.56	0.88	0.09	-	0.13
A148/08	DLB	-	-	-	-	-	-	-	-
A162/07	DLB	-	-	-	-	-	-	-	-
A190/03	DLB	0.59	0.51	0.78	0.48	0.77	0.01	0.20	0.07
A196/09	DLB	-	0.67	-	-	-	0.09	-	-
A204/07	DLB	1.41	0.73	-	0.45	1.15	0.09	-	0.04
A229/05	DLB	0.55	0.67	0.57	0.49	0.74	0	0.06	0.07
A231/06	DLB	0.34	0.2	-	0.17	0.53	-0.45	-	-0.28
A249/06	DLB	-	-	-	-	-	-	-	-
A273/05	DLB	0.87	0.88	-	0.61	0.94	0.14	-	0.17
A304/06	DLB	-	0.62	-	0.54	-	0.16	-	0.23
A335/08	DLB	0.65	0.62	-	0.35	0.81	0	-	-0.07
A336/99	DLB	0.68	0.35	0.55	0.4	0.83	-0.21	0.05	-0.01
C1007 01/176	DLB	0.91	0.33	0.63	0.12	0.96	-0.11	-0.05	-0.54
ST26/04	DLB	0.68	0.91	0.72	0.52	0.83	0.3	0.09	0.1
ST27/04	DLB	0.66	0.37	0.78	0.39	0.82	0.04	0.13	-0.03
ST28/04	DLB	0.53	0.36	0.64	0.27	0.72	-	0.14	-0.08
ST32/05	DLB	0.57	0.4	0.35	0.17	0.76	-0.15	-0.17	-0.28
A071/09	AD	1.04	0.48	1.21	0.69	1.02	-0.12	0.04	0.22
A108/09	AD	0.78	0.53	0.71	0.48	0.89	-0.02	-0.06	0.17
A120/09	AD	0.84	-	0.77	0.34	0.92	-	-0.06	0.02

CaseID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Normalised Btub	Residual PSD95	Residual SPP	Residual ZnT3
A147/10	AD	0.74	0.68	1.57	0.16	0.87	0.07	0.29	-0.3
A216/09	AD	0.34	-	1.53	0.22	0.53	-	0.65	-0.16
A267/09	AD	0.78	0.32	1.37	0.13	0.89	-0.06	0.22	-0.39
A349/08	AD	-	0.32	1.66	0.23	-	-0.28	-	-0.15
A350/09	AD	0.94	0.4	1.46	0.45	0.97	-0.15	0.17	0.15
A37/09	AD	0.85	1.07	0.53	0.5	0.93	0.3	-0.23	0.08
A371/08	AD	0.71	0.32	1.44	0.52	0.85	-0.07	0.30	0.1
A38/11	AD	0.77	0.68	1.47	0.18	0.89	0.24	0.23	-0.25
A61/09	AD	0.95	0.66	0.84	0.44	0.98	0.03	-0.08	0.14
A7/10	AD	0.86	0.93	0.57	0.51	0.93	0.24	-0.22	0.2
A76/09	AD	0.88	1.1	1.28	0.77	0.94	0.27	0.14	0.27
A8/10	AD	0.74	-	1.36	0.29	0.87	-	0.23	-0.04
A92/09	AD	1.09	1.3	0.95	0.58	1.04	0.34	-0.08	0.15

Residual and normalised protein ratios in BA9

Case ID	Diagnosis	SPP to BTUB	PSD to BTUB	ZnT3 to SPP	Normalised SPP to BTUB	Residual PSD to BTUB	Residual ZnT3 to SPP
A011/06	Control	0.87	1.31	1.03	-0.06	0.35	0.29
A047/02	Control	0.50	0.82	1.48	-0.30	0.06	0.54
A048/09	Control	1.40	0.69	1.88	0.14	0.07	0.47
A049/03	Control	0.46	0.58	1.94	-0.34	-0.04	0.59
A063/10	Control	1.39	0.52	1.27	0.14	0.07	0.33
A133/95	Control	0.66	0.41	0.90	-0.18	-0.14	0.07
A134/00	Control	0.38	1.47	1.14	-0.42	0.25	0.23
A136/10	Control	1.35	0.96	1.88	0.13	0.30	0.54
A153/01	Control	1.09	1.17	0.40	0.04	0.15	-0.13
A170/00	Control	1.02	3.81	0.48	0.01	0.67	-0.09
A185/04	Control	0.76	1.66	0.42	-0.12	0.47	-0.15
A219/97	Control	-	-	0.31	-	-	-0.15
A223/96	Control	-	0.73	-	-	-0.03	-
A239/95	Control	-	-	0.48	-	-	-0.07
A283/96	Control	0.59	0.54	0.41	-0.23	-0.09	-0.14
A308/09	Control	1.40	1.51	1.88	0.15	0.45	0.48
A31/96	Control	0.84	2.41	1.22	-0.08	0.62	0.26
A316/95	Control	1.61	0.55	0.60	0.21	-0.06	0.04
A320/94	Control	0.77	0.37	0.69	-0.11	0.02	0.06
A33/96	Control	0.61	0.24	0.42	-0.22	-0.28	-0.03
A346/95	Control	0.85	0.61	0.71	-0.07	-0.09	0.19
A359/08	Control	0.69	0.50	1.48	-0.16	-0.16	0.42
A401/97	Control	0.84	2.11	1.14	-0.07	0.55	0.42
A61/96	Control	-	-	-	-	-	-
A94/95	Control	1.10	1.33	1.04	0.04	0.31	0.35

Case ID	Diagnosis	SPP to BTUB	PSD to BTUB	ZnT3 to SPP	Normalised SPP to BTUB	Residual PSD to BTUB	Residual ZnT3 to SPP
20020080	PDD	0.93	0.59	0.64	-0.03	-0.11	0.13
20030004	PDD	0.91	0.60	1.06	-0.04	0.03	0.24
20030103	PDD	1.10	0.23	0.26	0.04	-0.46	-0.16
20030111	PDD	-	-	-	-	-	-
20030134	PDD	0.19	0.21	0.73	-0.73	-0.45	0.12
20040022	PDD	0.72	0.32	0.49	-0.14	-0.32	-0.13
20040076	PDD	1.22	1.33	0.56	0.09	0.25	0.01
20040105	PDD	0.73	1.04	0.69	-0.13	0.12	0.18
20050096	PDD	-	0.45	-	-	-0.17	-
20050099	PDD	0.85	0.26	0.57	-0.07	-0.26	0.13
A143/00	PDD	1.15	0.48	0.23	0.06	-0.04	-0.28
ST01/01	PDD	0.71	0.85	0.57	-0.15	0.08	-0.03
ST02/01	PDD	0.59	0.49	0.66	-0.23	-0.11	0.08
ST03/01	PDD	0.83	0.46	1.27	-0.08	-0.13	0.40
ST04/01	PDD	0.98	0.77	0.57	-0.01	-	-0.08
ST09/02	PDD	0.65	0.72	0.89	-0.19	0.21	0.12
ST10/02	PDD	0.45	0.73	0.97	-0.35	0.02	0.25
ST11/02	PDD	0.54	0.84	0.94	-0.27	0.22	0.18
ST12/02	PDD	0.39	0.42	-	-0.41	-0.20	-
ST13/02	PDD	0.42	0.26	1.41	-0.38	-0.41	0.31
ST14/02	PDD	0.52	0.63	0.28	-0.29	-0.05	-0.30
ST15/02	PDD	0.57	0.75	0.30	-0.25	0.23	-0.12
ST16/02	PDD	1.22	1.06	0.87	0.09	0.19	0.17
ST17/02	PDD	0.91	1.09	0.50	-0.04	0.13	-0.15
ST18/02	PDD	0.73	0.59	0.33	-0.14	-0.05	-0.31
ST19/02	PDD	0.29	0.37	0.59	-0.53	-0.26	0.10

Case ID	Diagnosis	SPP to BTUB	PSD to BTUB	ZnT3 to SPP	Normalised SPP to BTUB	Residual PSD to BTUB	Residual ZnT3 to SPP
ST20/02	PDD	0.49	1.37	0.85	-0.31	0.34	0.21
ST21/03	PDD	0.55	0.28	0.41	-0.26	-0.39	-0.12
ST22/02	PDD	0.31	-	0.75	-0.51	-	-0.05
ST23/03	PDD	1.00	0.46	1.09	0.00	-0.14	0.23
ST24/03	PDD	0.81	0.59	-	-0.09	-0.08	-
ST25/04	PDD	0.73	0.68	0.44	-0.14	-0.01	-0.05
ST29/04	PDD	0.66	0.60	0.40	-0.18	-0.03	-0.06
ST30/04	PDD	-	0.27	-	-	-0.39	-
027 93/1089	DLB	0.59	0.49	0.41	-0.23	-0.20	-0.10
036 93/1075	DLB	-	-	-	-	-	-
051 91/1249	DLB	0.74	0.19	0.24	-0.13	-0.33	-0.35
052 94/1224	DLB	0.63	0.80	0.59	-0.20	0.08	0.09
055 98/1226	DLB	0.59	0.33	0.33	-0.23	-0.27	-0.28
106 99/1109	DLB	0.98	0.68	0.28	-0.01	0.03	-0.38
20030007	DLB	1.51	0.91	0.22	0.18	0.08	-0.27
20030113	DLB	0.31	0.07	0.49	-0.51	-0.81	0.06
20040034	DLB	0.48	0.28	0.30	-0.32	-0.23	-0.07
20040085	DLB	-	-	0.31	-	-	-0.06
20050030	DLB	1.77	0.63	0.19	0.25	0.20	-0.28
20050040	DLB	-	-	0.34	-	-	-0.08
20060025	DLB	-	-	-	-	-	-
20070009	DLB	0.67	0.39	0.51	-0.18	-0.18	0.18
20070105	DLB	1.42	1.05	0.29	0.15	0.11	-0.10
20080083	DLB	0.82	0.49	0.54	-0.08	-0.18	0.18
20100575	DLB	1.33	0.25	0.41	0.12	-0.36	0.00
333 08/064	DLB	0.92	0.78	0.25	-0.04	0.05	-0.27

Case ID	Diagnosis	SPP to BTUB	PSD to BTUB	ZnT3 to SPP	Normalised SPP to BTUB	Residual PSD to BTUB	Residual ZnT3 to SPP
367 08/134	DLB	1.02	0.27	0.19	0.01	-0.12	-0.46
383 99/1147	DLB	0.51	0.64	0.55	-0.29	0.11	0.12
436 03/148	DLB	0.98	0.89	0.59	-0.01	0.29	0.09
439 00/1140	DLB	1.07	-	0.50	0.03	-	-0.15
470 01/156	DLB	0.73	0.29	0.25	-0.13	-0.19	-0.35
475 00/1108	DLB	1.14	0.57	0.36	0.06	-0.03	-0.16
495 01/172	DLB	1.00	0.39	0.70	0.00	0.12	0.03
550 02/021	DLB	0.64	0.30	0.52	-0.19	-0.24	-0.12
745 08/126	DLB	1.07	0.55	0.68	0.03	0.19	0.07
A014/07	DLB	1.13	0.83	0.65	0.05	0.06	-0.03
A028/10	DLB	-	-	-	-	-	-
A035/08	DLB	-	1.07	-	-	0.14	-
A040/10	DLB	-	-	-	-	-	-
A046/07	DLB	0.80	0.55	-	-0.10	0.02	-
A053/09	DLB	0.71	0.24	-	-0.15	-0.37	-
A055/09	DLB	-	0.35	-	-	-0.27	-
A072/09	DLB	-	-	-	-	-	-
A084/09	DLB	-	-	1.89	-	-	0.67
A092/07	DLB	-	0.50	-	-	-0.17	-
A109/01	DLB	-	1.07	-	-	0.10	-
A148/08	DLB	-	-	-	-	-	-
A162/07	DLB	-	-	-	-	-	-
A190/03	DLB	1.32	0.86	0.62	0.12	0.15	0.10
A196/09	DLB	-	-	-	-	-	-
A204/07	DLB	-	0.52	-	-	-0.16	-
A229/05	DLB	1.04	1.22	0.86	0.02	0.16	0.08

Case ID	Diagnosis	SPP to BTUB	PSD to BTUB	ZnT3 to SPP	Normalised SPP to BTUB	Residual PSD to BTUB	Residual ZnT3 to SPP
A231/06	DLB	-	0.59	-	-	-0.08	-
A249/06	DLB	-	-	-	-	-	-
A273/05	DLB	-	1.01	-	-	0.09	-
A304/06	DLB	-	-	-	-	-	-
A335/08	DLB	-	0.95	-	-	0.09	-
A336/99	DLB	0.81	0.51	0.73	-0.09	-0.15	-0.09
C1007 01/176	DLB	0.69	0.36	0.19	-0.16	-0.16	-0.53
ST26/04	DLB	1.06	1.34	0.72	0.02	0.38	0.12
ST27/04	DLB	1.18	0.56	0.50	0.07	0.15	-0.06
ST28/04	DLB	1.21	0.68	0.42	0.08	-	-0.14
ST32/05	DLB	0.61	0.70	0.49	-0.21	0.00	0.04
A071/09	AD	1.16	0.46	0.57	0.07	-0.24	0.05
A108/09	AD	0.91	0.68	0.68	-0.04	-0.01	0.02
A120/09	AD	0.92	-	0.44	-0.04	-	-0.06
A147/10	AD	2.12	0.92	0.10	0.33	0.10	-0.60
A216/09	AD	4.50	-	0.14	0.65	-	-0.46
A267/09	AD	1.76	0.41	0.09	0.24	-0.03	-0.64
A349/08	AD	-	-	0.14	-	-	-0.62
A350/09	AD	1.55	0.43	0.31	0.19	-0.22	-0.23
A37/09	AD	0.62	1.26	0.94	-0.21	0.27	0.22
A371/08	AD	2.03	0.45	0.36	0.31	0.00	-0.35
A38/11	AD	1.91	0.88	0.12	0.28	0.28	-0.53
A61/09	AD	0.88	0.69	0.52	-0.05	-0.05	0.00
A7/10	AD	0.66	1.08	0.89	-0.18	0.21	0.31
A76/09	AD	1.45	1.25	0.60	0.16	0.23	0.11
A8/10	AD	1.84	-	0.21	0.26	-	-0.31

Case ID	Diagnosis	SPP to BTUB	PSD to BTUB	ZnT3 to SPP	Normalised SPP to BTUB	Residual PSD to BTUB	Residual ZnT3 to SPP
A92/09	AD	0.87	1.19	0.61	-0.06	0.20	0.05

Residual and normalised protein values in BA24

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Normalised PSD95	Residual SPP	Residual ZnT3
A011/06	Control	1.46	0.42	0.96	0.79	0.27	0.65	0.94	0.39
A047/02	Control	-	-	-	-	-	-	-	-
A048/09	Control	1.08	-	0.98	1.43	0.07	-	1.14	0.55
A049/03	Control	0.60	0.98	1.95	1.09	-0.18	0.99	2.20	0.54
A063/10	Control	0.86	0.53	0.96	0.38	0.04	0.73	0.49	0.09
A133/95	Control	-	0.44	0.76	0.38	-	0.66	-0.14	-0.07
A134/00	Control	0.61	0.26	0.77	0.51	-0.18	0.51	0.38	0.13
A136/10	Control	1.06	0.40	0.99	1.34	0.14	0.63	0.74	0.66
A153/01	Control	2.04	0.71	0.90	0.47	0.35	0.84	1.19	0.07
A170/00	Control	0.48	0.24	0.77	0.28	-0.21	0.49	0.36	-0.23
A185/04	Control	1.50	-	0.80	0.37	0.21	-	0.02	0.00
A219/97	Control	-	-	-	-	-	-	0.00	-
A223/96	Control	1.37	-	0.87	0.31	0.18	-	0.91	-0.12
A239/95	Control	1.86	0.23	0.84	0.33	0.38	0.48	0.33	-0.04
A283/96	Control	0.95	0.44	0.54	0.48	0.02	0.66	-0.98	0.10
A308/09	Control	0.63	-	0.99	0.86	-0.16	-	1.02	0.23
A31/96	Control	0.43	0.53	0.62	0.39	-0.33	0.73	-0.77	-0.12
A316/95	Control	0.50	0.72	0.53	0.24	-0.26	0.85	-1.19	-0.16
A320/94	Control	-	-	0.88	0.42	-	-	-0.21	0.02
A33/96	Control	0.77	0.43	0.48	0.27	0.00	0.66	-1.84	0.11
A346/95	Control	0.58	0.17	0.45	0.31	-0.20	0.41	-1.35	0.07
A359/08	Control	0.68	-	0.73	-	-0.06	-	-	-
A401/97	Control	0.98	0.22	0.37	0.12	0.03	0.47	-2.20	-0.31
A61/96	Control	0.62	0.24	0.70	-	-0.17	0.49	-0.33	-
A94/95	Control	0.90	0.85	0.71	0.41	0.06	0.92	-0.26	0.15

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Normalised PSD95	Residual SPP	Residual ZnT3
20020080	PDD	1.02	0.65	0.73	0.35	0.05	0.81	0.09	0.00
20030004	PDD	0.89	0.51	0.76	0.47	0.06	0.71	-0.09	0.00
20030103	PDD	1.20	0.78	0.75	0.50	0.19	0.88	-0.02	0.27
20030111	PDD	0.82	0.27	0.67	0.33	-0.05	0.52	-0.44	0.12
20030134	PDD	0.65	0.38	0.57	0.35	-0.15	0.62	-0.94	-0.04
20040022	PDD	0.90	0.47	0.57	0.34	-0.01	0.69	-0.84	-0.10
20040076	PDD	1.06	1.60	0.49	0.57	0.06	1.26	-1.10	0.19
20040105	PDD	1.40	1.50	0.52	0.59	0.18	1.22	-0.87	0.22
20050096	PDD	0.93	0.87	0.44	0.37	0.01	0.93	-1.55	0.14
20050099	PDD	0.83	0.66	0.58	0.26	-0.04	0.81	-1.24	0.07
A143/00	PDD	-	-	-	-	-	-	-	-
ST01/01	PDD	0.47	0.18	0.73	0.54	-0.22	0.42	-0.05	0.17
ST02/01	PDD	0.79	0.59	0.78	0.48	-0.06	0.77	0.07	0.16
ST03/01	PDD	0.91	0.16	1.68	0.17	0.00	0.40	1.99	-0.31
ST04/01	PDD	0.93	0.45	1.79	0.35	0.08	0.67	-	-0.05
ST09/02	PDD	0.81	0.04	2.60	0.21	-0.05	0.20	2.60	-0.32
ST10/02	PDD	0.84	0.14	0.69	0.21	-0.04	0.37	-0.28	-0.20
ST11/02	PDD	0.91	0.36	0.68	0.20	0.07	0.60	-0.65	-0.36
ST12/02	PDD	0.99	0.63	-	0.52	0.11	0.79	-	0.21
ST13/02	PDD	0.50	0.34	0.61	0.42	-0.19	0.58	-0.54	-0.02
ST14/02	PDD	0.92	0.72	1.14	0.14	0.00	0.85	1.55	-0.42
ST15/02	PDD	0.63	0.10	0.44	0.10	-0.09	0.32	-2.60	-0.33
ST16/02	PDD	0.76	0.54	0.85	0.40	-0.08	0.73	0.54	0.03
ST17/02	PDD	0.77	0.57	0.64	0.27	-0.07	0.75	-0.38	-0.29
ST18/02	PDD	1.16	0.42	0.88	0.72	0.10	0.65	0.62	0.22
ST19/02	PDD	0.36	0.31	0.59	0.29	-0.33	0.56	-0.68	0.03
ST20/02	PDD	0.45	0.44	0.75	0.36	-0.24	0.66	-0.07	0.08

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Normalised PSD95	Residual SPP	Residual ZnT3
ST21/03	PDD	0.96	0.53	0.88	0.22	0.09	0.73	0.71	-0.17
ST22/02	PDD	0.44	0.21	0.54	0.48	-0.25	0.46	-0.91	-0.09
ST23/03	PDD	0.58	0.25	0.55	0.44	-0.13	0.50	-1.02	0.04
ST24/03	PDD	0.90	1.24	0.75	0.30	-0.01	1.11	0.12	-0.01
ST25/04	PDD	0.41	0.49	0.61	0.35	-0.28	0.70	-0.49	0.09
ST29/04	PDD	0.37	0.52	0.38	0.65	-0.32	0.72	-1.73	0.42
ST30/04	PDD	1.11	0.07	-	0.21	0.08	0.26	-	-0.24
027 93/1089	DLB	1.03	0.38	0.73	0.15	0.12	0.62	0.19	-0.29
036 93/1075	DLB	1.61	1.05	1.13	0.19	0.25	1.02	1.63	-0.15
051 91/1249	DLB	1.33	0.59	1.02	0.54	0.16	0.77	0.68	0.21
052 94/1224	DLB	0.84	0.50	0.90	0.33	-0.04	0.71	0.81	0.06
055 98/1226	DLB	1.07	1.07	0.96	0.54	0.07	1.03	1.10	0.13
106 99/1109	DLB	1.09	2.19	-	0.51	0.15	1.48	-	0.13
20030007	DLB	0.83	0.45	0.85	0.35	0.03	0.67	0.65	0.21
20030113	DLB	0.86	0.55	0.93	0.25	-0.03	0.74	0.44	-0.04
20040034	DLB	-	0.31	0.72	0.10	-	0.56	-0.46	-0.37
20040085	DLB	0.66	0.30	0.60	0.20	-0.14	0.55	-0.62	-0.07
20050030	DLB	-	0.43	1.08	0.13	-	0.66	1.06	-0.15
20050040	DLB	-	0.57	1.03	0.26	-	0.75	0.84	-0.03
20060025	DLB	-	0.70	-	-	-	0.84	-	-
20070009	DLB	0.87	1.01	0.94	0.29	-0.02	1.00	0.87	0.10
20070105	DLB	0.83	0.52	0.81	0.23	-0.04	0.72	0.57	-0.06
20080083	DLB	0.70	0.39	0.67	0.28	-0.04	0.62	-0.31	0.11
20100575	DLB	0.95	0.29	0.81	0.43	0.02	0.54	0.14	0.21
333 08/064	DLB	1.07	0.49	0.70	0.15	0.14	0.70	-0.12	-0.23
367 08/134	DLB	0.94	0.32	0.54	0.12	0.08	0.57	-1.99	-0.37
383 99/1147	DLB	-	0.78	-	0.25	-	0.88	-	0.08

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Normalised PSD95	Residual SPP	Residual ZnT3
436 03/148	DLB	-	0.81	0.94	0.39	-	0.90	0.41	0.08
439 00/1140	DLB	0.78	0.26	0.89	0.30	-0.07	0.51	0.16	-0.22
470 01/156	DLB	0.73	-	0.81	0.33	-0.10	-	-0.36	0.00
475 00/1108	DLB	0.98	-	0.61	0.12	0.10	-	-0.74	-0.39
495 01/172	DLB	0.73	0.38	0.71	0.18	-0.10	0.62	-1.29	-0.31
550 02/021	DLB	1.09	0.50	1.14	0.23	0.08	0.71	1.41	-0.31
745 08/126	DLB	1.24	0.30	1.41	0.32	0.20	0.55	1.84	-0.09
A014/07	DLB	-	-	-	-	-	-	-	-
A028/10	DLB	-	-	-	-	-	-	-	-
A035/08	DLB	-	0.62	-	-	-	0.79	-	-
A040/10	DLB	-	-	-	-	-	-	-	-
A046/07	DLB	-	-	-	-	-	-	-	-
A053/09	DLB	0.69	-	0.88	-	-0.12	-	0.46	-
A055/09	DLB	0.59	-	0.59	-	-0.12	-	-0.71	-
A072/09	DLB	-	-	-	-	-	-	-	-
A084/09	DLB	-	-	-	-	-	-	-	-
A092/07	DLB	-	0.52	-	-	-	0.72	-	-
A109/01	DLB	1.50	1.06	0.40	-	0.21	1.03	-1.48	-
A148/08	DLB	0.96	-	0.48	0.27	0.09	-	-1.14	0.02
A162/07	DLB	0.99	-	0.46	-	0.03	-	-1.41	-
A190/03	DLB	-	0.87	-	-	-	0.93	-	-
A196/09	DLB	-	-	-	-	-	-	-	-
A204/07	DLB	1.35	1.22	0.76	0.86	0.17	1.10	0.21	0.28
A229/05	DLB	-	0.63	-	-	-	0.79	-	-
A231/06	DLB	1.49	0.35	0.78	-	0.28	0.59	0.26	-
A249/06	DLB	1.68	-	0.64	1.02	0.26	-	-0.19	0.41
A273/05	DLB	1.16	0.58	0.65	-	0.10	0.76	-0.24	-

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Normalised PSD95	Residual SPP	Residual ZnT3
A304/06	DLB	1.04	0.62	0.60	-	0.13	0.79	-1.06	-
A335/08	DLB	1.05	0.52	0.63	0.35	0.06	0.72	-0.41	-0.09
A336/99	DLB	-	-	-	-	-	-	-	-
C1007 01/176	DLB	1.41	0.54	1.11	0.39	0.19	0.73	1.29	-0.01
ST26/04	DLB	0.86	0.42	0.86	0.18	-0.03	0.65	0.28	-0.21
ST27/04	DLB	0.84	0.99	0.94	0.85	-0.04	0.99	0.24	0.37
ST28/04	DLB	0.44	0.39	0.70	0.60	-0.25	0.62	-	0.27
ST32/05	DLB	0.46	0.22	0.60	0.21	-0.23	0.47	-0.51	-0.05
A071/09	AD	0.99	0.58	0.82	0.67	0.03	0.76	0.60	0.32
A108/09	AD	0.63	0.34	0.74	0.52	-0.09	0.58	0.05	0.13
A120/09	AD	0.63	0.43	0.84	0.09	-0.09	0.66	-0.16	-0.51
A147/10	AD	0.85	0.90	0.79	0.06	0.04	0.95	0.31	-0.58
A216/09	AD	0.73	0.45	0.65	0.32	-0.03	0.67	-0.57	0.16
A267/09	AD	0.71	0.70	0.51	0.08	-0.04	0.84	-1.63	-0.43
A349/08	AD	0.99	0.81	-	0.21	0.11	0.90	-	-0.20
A350/09	AD	1.21	-	1.17	0.52	0.19	-	1.73	0.34
A37/09	AD	0.96	1.92	1.06	0.45	0.02	1.39	1.35	0.16
A371/08	AD	0.69	0.44	0.68	0.48	-0.12	0.66	-0.81	-0.03
A38/11	AD	0.72	1.08	0.71	0.17	-0.03	1.04	-0.60	-0.24
A61/09	AD	0.91	0.82	0.88	0.07	0.07	0.91	0.98	-0.49
A7/10	AD	0.85	1.17	1.03	0.25	0.04	1.08	1.24	0.00
A76/09	AD	0.66	0.68	0.87	0.29	-0.14	0.82	0.77	0.13
A8/10	AD	0.70	-	0.84	0.25	-0.04	-	0.51	0.11
A92/09	AD	0.94	0.93	1.10	0.71	0.01	0.96	1.48	0.37

Residual and normalised protein ratios in BA24

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Normalised PSD95 to Btub	Residual ZnT3 to SPP
A011/06	Control	0.66	0.29	0.82	-0.18	0.54	1.30
A047/02	Control	-	-	-	-	-	0.00
A048/09	Control	0.91	-	1.46	-0.04	-	1.95
A049/03	Control	3.25	1.63	0.56	0.51	1.28	0.52
A063/10	Control	1.12	0.62	0.40	0.05	0.79	0.04
A133/95	Control	-	-	0.50	-	-	-0.33
A134/00	Control	1.26	0.43	0.66	0.10	0.65	-0.09
A136/10	Control	0.93	0.38	1.35	-0.03	0.61	2.16
A153/01	Control	0.44	0.35	0.52	-0.36	0.59	-0.14
A170/00	Control	1.60	0.50	0.36	0.21	0.71	-0.88
A185/04	Control	0.53	-	0.46	-0.27	-	-0.04
A219/97	Control	-	-	-	-	-	0.00
A223/96	Control	0.64	-	0.36	-0.20	-	-1.00
A239/95	Control	0.45	0.12	0.39	-0.35	0.35	-0.44
A283/96	Control	0.57	0.46	0.89	-0.25	0.68	1.09
A308/09	Control	1.57	-	0.87	0.20	-	1.14
A31/96	Control	1.44	1.23	0.63	0.16	1.11	0.30
A316/95	Control	1.06	1.44	0.45	0.03	1.20	-0.06
A320/94	Control	-	-	0.48	-	-	0.74
A33/96	Control	0.62	0.56	0.56	-0.21	0.75	1.24
A346/95	Control	0.78	0.29	0.69	-0.11	0.54	0.92
A359/08	Control	1.07	-	-	0.03	-	0.00
A401/97	Control	0.38	0.22	0.32	-0.42	0.47	-0.19
A61/96	Control	1.13	0.39	-	0.05	0.62	0.00
A94/95	Control	0.79	0.94	0.58	-0.10	0.97	0.71
20020080	PDD	0.72	0.64	0.48	-0.15	0.80	0.09

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Normalised PSD95 to Btub	Residual ZnT3 to SPP
20030004	PDD	0.85	0.57	0.62	-0.07	0.76	0.49
20030103	PDD	0.63	0.65	0.67	-0.20	0.81	1.36
20030111	PDD	0.82	0.33	0.49	-0.09	0.57	0.81
20030134	PDD	0.88	0.58	0.61	-0.06	0.76	0.64
20040022	PDD	0.63	0.52	0.60	-0.20	0.72	0.01
20040076	PDD	0.46	1.51	1.16	-0.34	1.23	1.43
20040105	PDD	0.37	1.07	1.13	-0.43	1.04	1.59
20050096	PDD	0.47	0.94	0.84	-0.33	0.97	1.69
20050099	PDD	0.70	0.80	0.45	-0.16	0.89	0.88
A143/00	PDD	-	-	-	-	-	0.00
ST01/01	PDD	1.55	0.38	0.74	0.19	0.62	0.58
ST02/01	PDD	0.99	0.75	0.62	-0.01	0.86	0.61
ST03/01	PDD	1.85	0.18	0.10	0.27	0.42	-1.80
ST04/01	PDD	1.92	0.48	0.20	0.28	0.70	0.00
ST09/02	PDD	3.21	0.05	0.08	0.51	0.22	-2.16
ST10/02	PDD	0.82	0.17	0.30	-0.09	0.41	-0.85
ST11/02	PDD	0.75	0.40	0.29	-0.13	0.63	-0.77
ST12/02	PDD	-	0.64	-	-	0.80	0.00
ST13/02	PDD	1.22	0.68	0.69	0.09	0.82	0.22
ST14/02	PDD	1.24	0.78	0.12	0.09	0.88	-1.69
ST15/02	PDD	0.70	0.16	0.23	-0.16	0.40	-0.41
ST16/02	PDD	1.12	0.71	0.47	0.05	0.84	-0.25
ST17/02	PDD	0.83	0.74	0.42	-0.08	0.86	-0.92
ST18/02	PDD	0.76	0.36	0.82	-0.12	0.60	0.67
ST19/02	PDD	1.64	0.86	0.49	0.21	0.93	0.25
ST20/02	PDD	1.67	0.98	0.48	0.22	0.99	0.14
ST21/03	PDD	0.92	0.55	0.25	-0.04	0.74	-1.19
ST22/02	PDD	1.23	0.48	0.89	0.09	0.69	0.27

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Normalised PSD95 to Btub	Residual ZnT3 to SPP
ST23/03	PDD	0.95	0.43	0.80	-0.02	0.66	0.77
ST24/03	PDD	0.83	1.38	0.40	-0.08	1.17	-0.52
ST25/04	PDD	1.49	1.20	0.57	0.17	1.09	0.41
ST29/04	PDD	1.03	1.41	1.71	0.01	1.19	-
ST30/04	PDD	-	0.06	-	-	0.25	0.00
027 93/1089	DLB	0.71	0.37	0.21	-0.15	0.61	-1.43
036 93/1075	DLB	0.70	0.65	0.17	-0.15	0.81	-1.50
051 91/1249	DLB	0.77	0.44	0.53	-0.12	0.67	0.96
052 94/1224	DLB	1.07	0.60	0.37	0.03	0.77	-0.27
055 98/1226	DLB	0.90	1.00	0.56	-0.05	1.00	0.12
106 99/1109	DLB	-	2.01	-	-	1.42	0.00
20030007	DLB	1.02	0.54	0.41	0.01	0.74	0.06
20030113	DLB	1.08	0.64	0.27	0.03	0.80	-0.22
20040034	DLB	-	-	0.14	-	-	-1.05
20040085	DLB	0.91	0.45	0.33	-0.04	0.67	-0.01
20050030	DLB	-	-	0.12	-	-	-1.09
20050040	DLB	-	-	0.25	-	-	-0.12
20060025	DLB	-	-	-	-	-	0.00
20070009	DLB	1.08	1.16	0.31	0.03	1.08	0.17
20070105	DLB	0.98	0.63	0.28	-0.01	0.79	-0.55
20080083	DLB	0.96	0.56	0.42	-0.02	0.75	0.33
20100575	DLB	0.85	0.31	0.53	-0.07	0.55	1.05
333 08/064	DLB	0.65	0.46	0.21	-0.18	0.68	-1.14
367 08/134	DLB	0.57	0.34	0.22	-0.24	0.58	-0.71
383 99/1147	DLB	-	-	-	-	-	0.00
436 03/148	DLB	-	-	0.41	-	-	0.46
439 00/1140	DLB	1.14	0.33	0.34	0.06	0.58	-0.61
470 01/156	DLB	1.11	-	0.41	0.05	-	0.19

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Normalised PSD95 to Btub	Residual ZnT3 to SPP
475 00/1108	DLB	0.62	-	0.20	-0.21	-	-1.30
495 01/172	DLB	0.97	0.52	0.25	-0.01	0.72	-0.49
550 02/021	DLB	1.05	0.46	0.20	0.02	0.68	-1.36
745 08/126	DLB	1.14	0.24	0.23	0.06	0.49	-0.74
A014/07	DLB	-	-	-	-	-	0.00
A028/10	DLB	-	-	-	-	-	0.00
A035/08	DLB	-	-	-	-	-	0.00
A040/10	DLB	-	-	-	-	-	0.00
A046/07	DLB	-	-	-	-	-	0.00
A053/09	DLB	1.28	-	-	0.11	-	0.00
A055/09	DLB	1.00	-	-	0.00	-	0.00
A072/09	DLB	-	-	-	-	-	0.00
A084/09	DLB	-	-	-	-	-	0.00
A092/07	DLB	-	-	-	-	-	0.00
A109/01	DLB	0.27	0.71	-	-0.57	0.84	0.00
A148/08	DLB	0.50	-	0.56	-0.30	-	0.44
A162/07	DLB	0.46	-	-	-0.33	-	0.00
A190/03	DLB	-	-	-	-	-	0.00
A196/09	DLB	-	-	-	-	-	0.00
A204/07	DLB	0.56	0.90	1.13	-0.25	0.95	1.19
A229/05	DLB	-	-	-	-	-	0.00
A231/06	DLB	0.52	0.23	-	-0.28	0.48	0.00
A249/06	DLB	0.38	-	1.59	-0.42	-	1.50
A273/05	DLB	0.56	0.50	-	-0.25	0.71	0.00
A304/06	DLB	0.58	0.60	-	-0.24	0.77	0.00
A335/08	DLB	0.60	0.50	0.56	-0.22	0.70	-0.35
A336/99	DLB	-	-	-	-	-	0.00
C1007 01/176	DLB	0.79	0.38	0.35	-0.10	0.62	-0.67

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Normalised PSD95 to Btub	Residual ZnT3 to SPP
ST26/04	DLB	1.00	0.49	0.21	0.00	0.70	-1.24
ST27/04	DLB	1.12	1.18	0.90	0.05	1.09	1.80
ST28/04	DLB	1.59	0.89	0.86	0.20	0.94	0.00
ST32/05	DLB	1.30	0.48	0.35	0.12	0.69	-0.30
A071/09	AD	0.83	0.59	0.82	-0.08	0.77	1.00
A108/09	AD	1.17	0.54	0.70	0.07	0.73	0.35
A120/09	AD	1.33	0.68	0.11	0.12	0.83	-1.59
A147/10	AD	0.93	1.06	0.08	-0.03	1.03	-1.95
A216/09	AD	0.89	0.62	0.49	-0.05	0.79	0.85
A267/09	AD	0.72	0.99	0.16	-0.14	0.99	-0.96
A349/08	AD	-	0.82	-	-	0.90	0.00
A350/09	AD	0.97	-	0.44	-0.01	-	-0.17
A37/09	AD	1.10	2.00	0.42	0.04	1.41	-0.38
A371/08	AD	0.99	0.64	0.71	-0.01	0.80	0.55
A38/11	AD	0.99	1.50	0.24	-0.01	1.22	-0.46
A61/09	AD	0.97	0.90	0.08	-0.01	0.95	-
A7/10	AD	1.21	1.38	0.24	0.08	1.17	-0.81
A76/09	AD	1.32	1.03	0.33	0.12	1.02	-0.64
A8/10	AD	1.20	-	0.30	0.08	-	-0.58
A92/09	AD	1.17	0.99	0.65	0.07	0.99	0.38

Residual and normalised protein values in BA40

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Residual PSD95	Residual ZnT3
A011/06	Control	1.02	-	1.00	0.09	0.16	-	-0.50
A047/02	Control	1.07	-	1.14	0.17	0.26	-	-0.28
A048/09	Control	0.91	0.40	0.71	0.12	0.04	-0.18	-0.37
A049/03	Control	1.34	0.53	1.25	0.23	0.46	-0.11	-0.11
A063/10	Control	-	0.23	0.66	0.05	-	-0.22	-0.67
A133/95	Control	0.86	0.04	0.80	0.30	0.03	-0.59	0.04
A134/00	Control	0.85	0.98	0.89	-	0.03	0.06	-
A136/10	Control	1.14	1.01	1.09	0.71	0.34	0.27	0.46
A153/01	Control	0.82	2.01	1.13	0.49	-0.13	0.49	0.14
A170/00	Control	0.85	0.98	0.76	0.23	-0.13	0.07	-0.18
A185/04	Control	0.80	0.82	1.15	1.12	-0.07	0.12	0.61
A219/97	Control	1.05	0.85	0.66	0.61	0.14	0.18	0.39
A223/96	Control	1.22	2.00	1.66	0.53	0.35	0.50	0.19
A239/95	Control	0.86	0.87	0.80	1.30	-0.02	0.11	0.65
A283/96	Control	1.38	0.82	0.92	0.43	0.48	0.05	0.14
A308/09	Control	0.87	0.25	-	0.16	-0.12	-0.28	-0.22
A31/96	Control	0.80	0.83	0.63	0.41	-0.16	0.11	0.17
A316/95	Control	0.90	1.28	0.96	3.68	0.03	0.30	1.09
A320/94	Control	0.84	0.64	1.40	-	-0.06	0.17	-
A33/96	Control	1.01	0.37	0.96	0.46	0.27	-0.10	0.29
A346/95	Control	1.18	0.83	0.61	0.48	0.35	0.02	0.16
A359/08	Control	0.90	0.56	-	0.10	0.03	-0.13	-0.51
A401/97	Control	0.99	0.69	0.77	0.47	0.15	0.02	0.22
A61/96	Control	0.80	0.90	0.78	1.30	-0.20	0.10	0.62
A94/95	Control	0.94	1.05	0.84	-	0.07	0.18	-
A143/00	PDD	-	-	-	-	-	-	-
20020080	PDD	1.69	1.24	1.33	0.71	0.73	0.22	0.33
20030004	PDD	1.22	0.78	0.94	0.53	0.25	0.09	0.28
20030103	PDD	0.82	0.71	0.85	-	-0.11	0.00	-
20030111	PDD	0.79	0.27	0.65	0.16	-0.08	-0.29	-0.26
20030134	PDD	0.57	1.11	0.51	0.40	-0.35	0.24	0.14
20040022	PDD	0.75	-	0.74	0.75	-0.13	-	0.39
20040076	PDD	1.42	-	0.75	0.48	0.51	-	0.16
20040105	PDD	1.56	-	0.86	0.29	0.58	-	-0.08
20050096	PDD	1.02	1.02	1.02	0.99	0.09	0.17	0.51
20050099	PDD	0.70	0.50	0.73	0.19	-0.10	-0.03	-0.12
ST01/01	PDD	1.20	0.41	0.99	0.18	0.35	-0.23	-0.25
ST02/01	PDD	1.04	0.95	0.82	0.27	0.19	0.15	-0.04
ST03/01	PDD	0.70	0.59	1.00	0.45	-0.22	-0.06	0.18
ST04/01	PDD	1.09	-	0.73	0.22	0.26	-	-
ST09/02	PDD	1.06	0.30	0.70	0.34	0.18	-0.16	0.16

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Residual PSD95	Residual ZnT3
ST10/02	PDD	0.92	1.08	1.16	0.79	0.06	0.17	0.40
ST11/02	PDD	1.14	0.93	1.20	0.37	0.21	0.22	0.16
ST12/02	PDD	1.06	1.22	0.93	0.18	0.19	0.25	-0.24
ST13/02	PDD	0.89	0.61	0.83	0.45	0.02	-0.07	0.16
ST14/02	PDD	0.63	-	1.07	0.09	-0.26	-	-0.55
ST15/02	PDD	1.10	0.48	1.38	0.06	0.29	-0.02	-0.60
ST16/02	PDD	0.60	0.45	0.84	0.31	-0.27	-0.19	-0.01
ST17/02	PDD	-	1.06	0.91	0.36	-	0.11	0.01
ST18/02	PDD	0.97	1.50	0.96	0.87	0.09	0.38	0.45
ST19/02	PDD	0.50	1.40	0.94	0.49	-0.34	0.33	0.20
ST20/02	PDD	1.40	1.27	0.82	0.29	0.57	0.30	-0.01
ST21/03	PDD	1.32	1.15	0.80	0.17	0.47	0.21	-0.27
ST22/02	PDD	0.98	1.21	0.94	0.43	0.06	0.23	0.13
ST23/03	PDD	1.03	0.43	0.93	0.33	0.17	-0.18	0.04
ST24/03	PDD	0.79	-	0.68	0.24	-0.02	-	-0.12
ST25/04	PDD	1.12	0.86	1.39	-	0.30	0.06	-
ST29/04	PDD	1.43	1.09	0.92	0.55	0.62	0.20	0.26
ST30/04	PDD	0.44	0.81	0.88	0.40	-0.38	0.06	0.12
A014/07	DLB	-	-	-	-	-	-	-
A028/10	DLB	-	-	-	-	-	-	-
A035/08	DLB	-	-	-	-	-	-	-
A040/10	DLB	-	-	-	-	-	-	-
A046/07	DLB	-	1.36	-	0.39	-	0.39	0.17
A053/09	DLB	-	-	-	-	-	-	-
A055/09	DLB	-	-	-	-	-	-	-
A072/09	DLB	-	-	-	-	-	-	-
A084/09	DLB	-	-	-	-	-	-	-
A092/07	DLB	-	-	-	-	-	-	-
A109/01	DLB	-	-	-	-	-	-	-
A148/08	DLB	-	-	-	0.26	-	-	-0.12
A162/07	DLB	-	-	-	0.33	-	-	0.02
A190/03	DLB	-	-	-	-	-	-	-
A196/09	DLB	-	-	-	-	-	-	-
A204/07	DLB	-	-	-	-	-	-	-
A229/05	DLB	-	-	-	-	-	-	-
A231/06	DLB	-	-	-	-	-	-	-
A249/06	DLB	-	-	-	0.41	-	-	0.06
A273/05	DLB	-	1.12	-	0.18	-	0.14	-0.29
A304/06	DLB	-	-	-	-	-	-	-
A335/08	DLB	-	-	-	0.30	-	-	-0.06
A336/99	DLB	-	0.54	-	0.32	-	-0.14	-0.01
20030007	DLB	0.82	0.55	1.07	0.37	0.01	-0.15	0.04
20030113	DLB	0.84	0.63	0.80	0.18	-0.06	0.06	-0.14

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Residual PSD95	Residual ZnT3
20040034	DLB	0.83	0.33	1.07	0.14	-0.09	-0.16	-0.25
20040085	DLB	0.74	0.26	0.92	0.20	-0.16	-0.34	-0.19
20050030	DLB	0.89	0.27	0.75	0.08	0.11	-0.15	-0.44
20050040	DLB	1.00	0.24	1.04	0.13	0.08	-0.20	-0.24
20060025	DLB	0.90	0.81	0.81	0.35	-0.01	0.00	0.01
20070009	DLB	0.57	0.40	0.96	0.17	-0.36	-0.18	-0.22
20070105	DLB	0.90	0.95	1.01	0.32	-0.05	0.05	-0.04
20080083	DLB	1.04	0.47	0.88	0.29	0.17	-0.20	-0.06
20100575	DLB	0.88	0.36	0.79	0.26	-0.02	-0.19	-0.03
027 93/1089	DLB	0.73	0.68	-	0.48	-0.08	-0.08	0.15
036 93/1075	DLB	0.85	-	0.89	-	0.02	-	-
051 91/1249	DLB	0.58	0.44	0.56	0.19	-0.28	-0.02	-0.07
052 94/1224	DLB	0.44	0.83	0.82	0.26	-0.42	0.06	-0.07
055 98/1226	DLB	-	-	-	-	-	-	-
106 99/1109	DLB	0.86	0.10	0.60	0.13	0.05	-0.52	-0.36
333 08/064	DLB	0.84	0.27	0.58	0.19	0.03	-0.35	-0.22
367 08/134	DLB	0.71	0.72	-	0.15	-0.06	0.22	-0.13
383 99/1147	DLB	1.43	0.53	0.91	0.31	0.66	-0.02	0.09
436 03/148	DLB	0.75	0.73	-	0.50	-0.16	0.14	0.32
439 00/1140	DLB	0.81	0.36	0.65	0.15	-0.11	-0.10	-0.19
470 01/156	DLB	0.49	0.57	0.74	0.31	-0.35	0.05	0.12
475 00/1108	DLB	0.78	-	0.78	0.09	-0.05	-	-0.51
495 01/172	DLB	1.23	0.69	0.82	0.18	0.41	0.26	0.00
550 02/021	DLB	0.87	0.43	0.70	0.24	-0.04	-0.10	-0.03
745 08/126	DLB	0.31	0.69	0.66	0.31	-0.60	0.20	0.18
C1007 01/176	DLB	0.59	1.21	0.86	0.29	-0.27	0.33	0.04
ST26/04	DLB	0.81	-	0.96	0.35	0.02	-	0.11
ST27/04	DLB	0.94	0.20	-	0.42	0.07	-0.22	0.28
ST28/04	DLB	0.77	0.26	0.95	-	-0.04	-	-
ST32/05	DLB	-	0.37	0.91	0.11	-	-0.26	-0.46
A071/09	AD	0.53	0.22	0.57	0.08	-0.34	-0.44	-0.64
A108/09	AD	0.74	0.55	1.01	0.38	-0.10	-0.12	0.08
A120/09	AD	0.58	0.38	0.79	0.17	-0.25	-0.07	-0.12
A147/10	AD	0.22	0.28	0.31	0.23	-0.61	-0.35	-0.15
A216/09	AD	0.47	-	0.52	0.07	-0.34	-	-0.60
A267/09	AD	0.57	0.37	0.50	-	-0.22	-0.09	-
A349/08	AD	0.39	0.32	0.59	0.28	-0.43	-0.33	-0.08
A350/09	AD	0.40	-	0.60	0.38	-0.32	-	0.08
A37/09	AD	0.43	-	0.71	-	-0.38	-	-
A371/08	AD	0.49	0.29	0.58	0.33	-0.37	-0.18	0.14
A38/11	AD	0.32	0.32	0.44	0.29	-0.62	-0.16	0.08
A61/09	AD	0.69	-	1.37	0.47	0.01	-	0.14

Case ID	Diagnosis	Betatubulin	PSD95	SPP	ZnT3	Residual Btub	Residual PSD95	Residual ZnT3
A7/10	AD	1.12	-	0.33	0.17	0.28	-	-0.26
A76/09	AD	0.41	0.90	0.60	0.56	-0.32	0.06	0.23
A8/10	AD	0.43	0.50	0.51	0.19	-0.29	-0.16	-0.22
A92/09	AD	0.47	-	0.93	0.33	-0.34	-	0.00

Residual and normalised protein ratios in BA40

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Residual PSD95 to Btub	Residual ZnT3 to SPP
A011/06	Control	0.98	-	0.09	-0.01	-	-0.52
A047/02	Control	1.07	-	0.15	0.03	-	-0.30
A048/09	Control	0.78	0.44	0.17	-0.11	-0.24	-0.39
A049/03	Control	0.93	0.40	0.18	-0.03	-0.29	-0.36
A063/10	Control	-	-	0.08	-	-	-0.59
A133/95	Control	0.93	0.05	0.38	-0.03	-0.67	-0.05
A134/00	Control	1.05	1.15	-	0.02	0.07	-
A136/10	Control	0.96	0.89	0.65	-0.02	0.11	0.34
A153/01	Control	1.38	2.45	0.43	0.14	0.56	0.01
A170/00	Control	0.89	1.15	0.30	-0.05	0.08	0.01
A185/04	Control	1.44	1.03	0.97	0.16	0.13	0.37
A219/97	Control	0.63	0.81	0.92	-0.20	0.06	0.49
A223/96	Control	1.36	1.64	0.32	0.13	0.29	-0.12
A239/95	Control	0.93	1.01	1.63	-0.03	0.10	0.74
A283/96	Control	0.67	0.59	0.47	-0.18	-0.16	0.05
A308/09	Control	-	0.29	-	-	-0.33	-
A31/96	Control	0.79	1.04	0.65	-0.10	0.13	0.19
A316/95	Control	1.07	1.42	3.83	0.03	0.27	0.96
A320/94	Control	1.67	0.76	-	0.22	0.13	-
A33/96	Control	0.95	0.37	0.48	-0.02	-0.21	0.21
A346/95	Control	0.52	0.70	0.79	-0.29	-0.13	0.27
A359/08	Control	-	0.62	-	-	-0.17	-
A401/97	Control	0.78	0.70	0.61	-0.11	-0.06	0.16
A61/96	Control	0.98	1.13	1.67	-0.01	0.13	0.60
A94/95	Control	0.89	1.12	-	-0.05	0.13	-
A143/00	PDD	-	-	-	-	-	-
20020080	PDD	0.79	0.73	0.53	-0.10	-0.11	0.11
20030004	PDD	0.77	0.64	0.56	-0.11	-0.09	0.28
20030103	PDD	1.04	0.87	-	0.02	0.00	-
20030111	PDD	0.82	0.34	0.25	-0.08	-0.32	-0.23
20030134	PDD	0.89	1.95	0.78	-0.05	0.49	0.27
20040022	PDD	0.99	-	1.01	-0.01	-	0.38
20040076	PDD	0.52	-	0.64	-0.28	-	0.19
20040105	PDD	0.55	-	0.34	-0.26	-	-0.09
20050096	PDD	1.00	1.00	0.98	0.00	0.07	0.37
20050099	PDD	1.04	0.71	0.26	0.02	0.01	-0.21
ST01/01	PDD	0.83	0.34	0.18	-0.08	-0.37	-0.21
ST02/01	PDD	0.79	0.91	0.33	-0.10	0.04	-0.10
ST03/01	PDD	1.43	0.84	0.45	0.15	0.00	0.03
ST04/01	PDD	0.67	-	0.30	-0.17	-	0.01

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Residual PSD95 to Btub	Residual ZnT3 to SPP
ST09/02	PDD	0.66	0.28	0.49	-0.18	-0.28	0.06
ST10/02	PDD	1.26	1.17	0.68	0.10	0.13	0.21
ST11/02	PDD	1.05	0.82	0.31	0.02	0.06	0.02
ST12/02	PDD	0.88	1.15	0.19	-0.06	0.13	-0.19
ST13/02	PDD	0.93	0.69	0.54	-0.03	-0.11	0.26
ST14/02	PDD	1.70	-	0.08	0.23	-	-0.70
ST15/02	PDD	1.25	0.44	0.04	0.10	-0.15	-0.84
ST16/02	PDD	1.40	0.75	0.37	0.15	-0.08	-0.06
ST17/02	PDD	-	-	0.40	-	-	-0.02
ST18/02	PDD	0.99	1.55	0.91	0.00	0.31	0.34
ST19/02	PDD	1.88	2.80	0.52	0.27	0.73	0.24
ST20/02	PDD	0.59	0.91	0.35	-0.23	0.04	0.07
ST21/03	PDD	0.61	0.87	0.21	-0.22	-0.02	-0.15
ST22/02	PDD	0.96	1.23	0.46	-0.02	0.16	0.19
ST23/03	PDD	0.90	0.42	0.35	-0.04	-0.28	0.08
ST24/03	PDD	0.86	-	0.35	-0.07	-	-0.07
ST25/04	PDD	1.24	0.77	-	0.09	-0.07	-
ST29/04	PDD	0.64	0.76	0.60	-0.19	-0.05	0.30
ST30/04	PDD	2.00	1.84	0.45	0.30	0.43	0.04
A014/07	DLB	-	-	-	-	-	-
A028/10	DLB	-	-	-	-	-	-
A035/08	DLB	-	-	-	-	-	-
A040/10	DLB	-	-	-	-	-	-
A046/07	DLB	-	-	-	-	-	-
A053/09	DLB	-	-	-	-	-	-
A055/09	DLB	-	-	-	-	-	-
A072/09	DLB	-	-	-	-	-	-
A084/09	DLB	-	-	-	-	-	-
A092/07	DLB	-	-	-	-	-	-
A109/01	DLB	-	-	-	-	-	-
A148/08	DLB	-	-	-	-	-	-
A162/07	DLB	-	-	-	-	-	-
A190/03	DLB	-	-	-	-	-	-
A196/09	DLB	-	-	-	-	-	-
A204/07	DLB	-	-	-	-	-	-
A229/05	DLB	-	-	-	-	-	-
A231/06	DLB	-	-	-	-	-	-
A249/06	DLB	-	-	-	-	-	-
A273/05	DLB	-	-	-	-	-	-
A304/06	DLB	-	-	-	-	-	-
A335/08	DLB	-	-	-	-	-	-
A336/99	DLB	-	-	-	-	-	-

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Residual PSD95 to Btub	Residual ZnT3 to SPP
20030007	DLB	1.30	0.67	0.35	0.12	-0.15	0.07
20030113	DLB	0.95	0.75	0.23	-0.02	0.03	-0.27
20040034	DLB	1.29	0.40	0.13	0.11	-0.21	-0.36
20040085	DLB	1.24	0.35	0.22	0.09	-0.34	-0.28
20050030	DLB	0.84	0.30	0.11	-0.07	-0.23	-0.45
20050040	DLB	1.04	0.24	0.13	0.02	-0.31	-0.38
20060025	DLB	0.90	0.90	0.43	-0.05	-0.03	0.01
20070009	DLB	1.68	0.70	0.18	0.23	-0.06	-0.37
20070105	DLB	1.12	1.06	0.32	0.05	0.03	-0.12
20080083	DLB	0.85	0.45	0.33	-0.07	-0.30	0.04
20100575	DLB	0.90	0.41	0.33	-0.05	-0.25	-0.10
027 93/1089	DLB	-	0.93	-	-	-0.02	-
036 93/1075	DLB	1.05	-	-	0.02	-	-
051 91/1249	DLB	0.97	0.76	0.34	-0.02	0.08	-0.09
052 94/1224	DLB	1.86	1.89	0.32	0.27	0.44	-0.12
055 98/1226	DLB	-	-	-	-	-	-
106 99/1109	DLB	0.70	0.12	0.22	-0.16	-0.58	-0.14
333 08/064	DLB	0.69	0.32	0.33	-0.16	-0.38	0.04
367 08/134	DLB	-	1.01	-	-	0.26	-
383 99/1147	DLB	0.64	0.37	0.34	-0.20	-0.24	0.06
436 03/148	DLB	-	0.97	-	-	0.17	-
439 00/1140	DLB	0.81	0.45	0.23	-0.09	-0.13	-0.26
470 01/156	DLB	1.51	1.16	0.42	0.18	0.27	0.00
475 00/1108	DLB	1.00	-	0.12	0.00	-	-0.41
495 01/172	DLB	0.67	0.56	0.22	-0.18	0.06	-0.28
550 02/021	DLB	0.81	0.50	0.34	-0.09	-0.15	-0.09
745 08/126	DLB	2.13	2.23	0.47	0.33	0.75	0.20
C1007 01/176	DLB	1.47	2.07	0.34	0.17	0.58	-0.09
ST26/04	DLB	1.19	-	0.36	0.07	-	-0.06
ST27/04	DLB	-	0.21	-	-	-0.32	-
ST28/04	DLB	1.23	0.34	-	0.09	-	-
ST32/05	DLB	-	-	0.12	-	-	-0.39
A071/09	AD	1.08	0.42	0.14	0.03	-0.35	-0.47
A108/09	AD	1.36	0.74	0.38	0.14	-0.09	0.10
A120/09	AD	1.36	0.66	0.22	0.13	0.02	-0.14
A147/10	AD	1.41	1.27	0.74	0.15	0.17	0.40
A216/09	AD	1.11	-	0.13	0.04	-	-0.34
A267/09	AD	0.88	0.65	-	-0.06	0.00	-
A349/08	AD	1.51	0.82	0.47	0.18	-0.07	0.20
A350/09	AD	1.50	-	0.63	0.18	-	0.33
A37/09	AD	1.65	-	-	0.22	-	-
A371/08	AD	1.18	0.59	0.57	0.07	-0.05	0.13

Case ID	Diagnosis	SPP to Btub	PSD95 to Btub	ZnT3 to SPP	Normalised SPP to Btub	Residual PSD95 to Btub	Residual ZnT3 to SPP
A38/11	AD	1.38	1.00	0.66	0.14	0.17	0.35
A61/09	AD	1.99	-	0.34	0.30	-	0.06
A7/10	AD	0.29	-	0.52	-0.53	-	0.24
A76/09	AD	1.46	2.20	0.93	0.17	0.51	0.35
A8/10	AD	1.19	1.16	0.37	0.07	0.13	0.10
A92/09	AD	1.98	-	0.35	0.30	-	-0.07